

**SYNTHESIS OF SMALL MOLECULE PEPTIDOMIMETICS AND
THEIR CONFORMATIONALLY CONSTRAINED ANALOGUES AS
POTENTIAL HIV PROTEASE INHIBITORS**

A Thesis Submitted in Partial

Fulfillment of the Requirements

For the Degree of

DOCTOR OF PHILOSOPHY

By

E.N. PRABHAKARAN

To

**THE DEPARTMENT OF CHEMISTRY
INDIAN INSTITUTE OF TECHNOLOGY
KANPUR**

June 2000

DEDICATED

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STATEMENT

I hereby declare that the matter embodied in this thesis is the result of investigations carried out by me in the Department of Chemistry, Indian Institute of Technology, Kanpur, INDIA, under the supervision of Prof. Javed Iqbal.

In keeping with the general practice of reporting scientific observations, due acknowledgements have been made wherever the work described is based on findings of other investigators.

Kanpur
June, 2000


(E.N. Prabhakaran)

DEPARTMENT OF CHEMISTRY
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INDIA

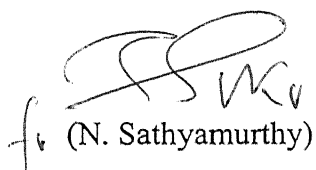
CERTIFICATE

This is to certify that Mr. E.N. Prabhakaran has satisfactorily completed all the courses required for the Ph.D. programme.

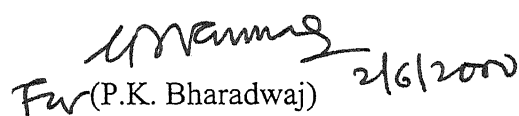
These courses include:

CHM 602 ADVANCED ORGANIC CHEMISTRY II
CHM 611 PHYSICAL ORGANIC CHEMISTRY
CHM 631 APPLICATION OF MODERN INSTRUMENTAL METHODS
CHM 664 MODERN PHYSICAL METHODS IN CHEMISTRY
CHM 646 BIO-ORGANIC CHEMISTRY
CHM 668 ADVANCED MAIN GROUP CHEMISTRY
CHM 800 GENERAL SEMINAR
CHM 801 GRADUATE SEMINAR
CHM 900 POST GRADUATE RESEARCH

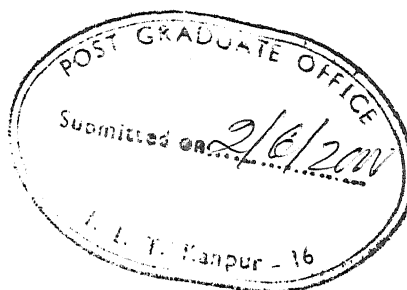
Mr. E.N. Prabhakaran successfully completed his Ph.D. qualifying examination in November 1995.


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For (P.K. Bharadwaj) 21/6/2000

Convenor,
Department Post Graduate Committee,
Department of Chemistry
I.I.T. Kanpur



Certificate II

This is to certify that the work carried out in the thesis entitled "*Synthesis of small molecule peptidomimetics and their conformationally constrained analogues as potential HIV protease inhibitors*" has been carried out by Mr. E. N. Prabhakaran under my supervision and the same has not been submitted elsewhere for a degree.

Hyderabad
May 31, 2000

A handwritten signature in cursive script that reads "Javed Iqbal".

Javed Iqbal
Professor,
Department of Chemistry
IIT Kanpur

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During the *Pilgrimage* towards higher living and learning in life, many *idols* have fascinated and shaped my thoughts, actions and deeds, towards whatever humble targets I have been able to reach thus far. The following is *only a few* of those who have been instrumental in such a process. I bow to one and all of them, in gratitude.

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Thanks to all my *Triplicane friends* and friends of Kamakala Kameshwarar koil. Thanks to all my school teachers, especially Mrs. Nagarathinam, Mrs. Leela, Mrs. Uma Parvathy, Mrs. Kaveri Padmanaban, Mr. Umesh and Dr. Santhanam whose ideals and memories shall remain to guide me throughout my life.

My thanks, filled with love for my sisters, Choti, Dubie, Pushki, Iffi, Shalu, Meenakshi, Huma, Khalida, Roopa and brothers Rajesh.V., Rajesh.S, Venkatraman, Nagarajan, Kamlesh, Bahadur, for giving all the love and encouragement during my stay at IIT K. My love for them, *forever*.

I take this opportunity to thank my past and present lab mates for being what they were, during the process of my post graduate life at IIT Kanpur. Special Thanks to *late* Asit

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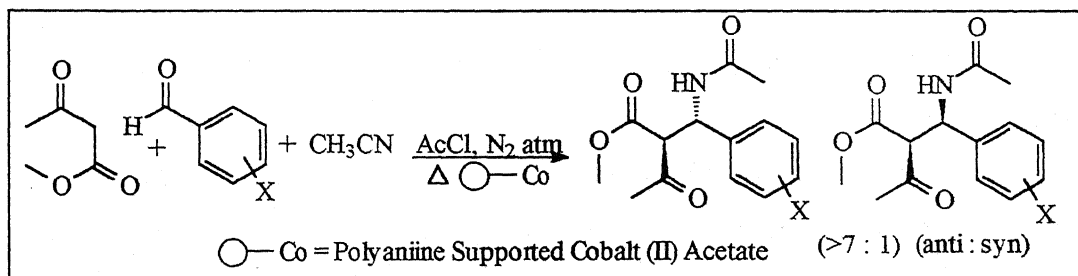
The work presented in the thesis entitled "**Synthesis of Small Molecule Peptidomimetics and Their Conformationally Constrained Analogues as Potential HIV Protease Inhibitors**" is summarised in the following pages. The thesis has been divided into two parts. The first part deals about the synthesis of small molecule peptidomimetics incorporating novel isosteres, for better binding at the HIV Protease active site. The second part is about the synthesis of conformationally constrained peptide analogues. Each part has been divided into two sections. Each section follows an introduction for a better understanding of the section.

PART A

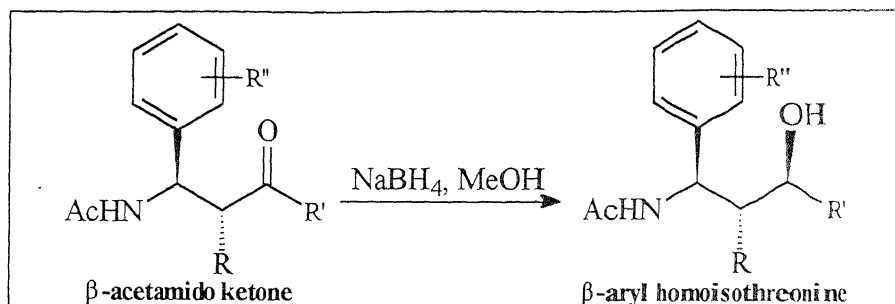
Section I

POLYANILINE SUPPORTED COBALT ACETATE CATALYSED THREE-COMPONENT COUPLING: SYNTHESIS OF β -AMINO ACID DERIVATIVES AS STRUCTURAL ANALOGUES OF HIV PR INHIBITORS

This section describes a *Polymer-Supported, Multi Component Coupling* synthetic methodology, for the synthesis of a library of small molecule β -amino acid derivatives containing novel α -acetyl- / α -(1-hydroxyethyl)-carbonyl isosteres as potential mechanism based inhibitors of aspartyl proteases.



This simple methodology involves the coupling of a ketone and an aldehyde with acetonitrile, in the presence of polyaniine supported cobalt (II) acetate (PASCOA), and acetylchloride to provide β -acetamido ketones containing the α -acetylcarbonyl isostere.



Reduction of these β -acetamido ketones led to the synthesis of α -(1-hydroxyethyl)-carbonyl isostere containing β -aryl homoisothreonine derivatives. These β -amino acid derivatives can be selectively deprotected at the N- and C-termini, for incorporation as part of peptide systems.

The advantages in this synthetic methodology are its simple, non-aqueous purification procedure for isolation of the β -amino acid derivatives, circumventing the need for column chromatography; the β -acetamido ketones are synthesised in high diastereomeric purity (anti : syn - >7 : 1); the β -aryl homoisothreonine derivatives are synthesised almost exclusively as the 1,3-syn diastereomeric products. To our knowledge, this is the first report of its kind for the synthesis of a library of β -acetamido ketone derivatives.

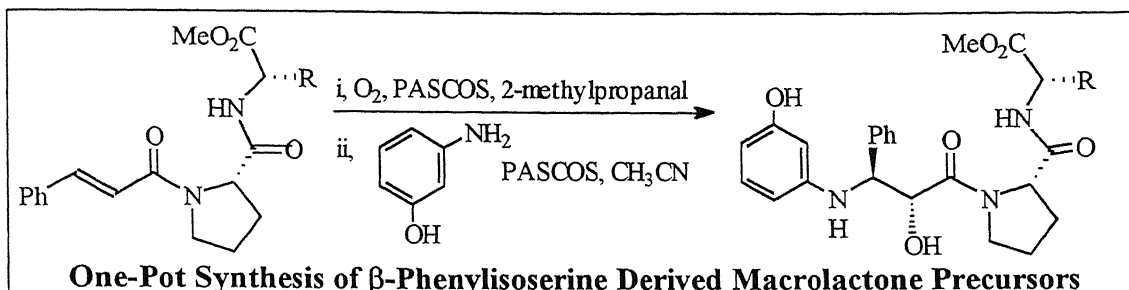
These small molecule peptides are acyclic structural analogues of the potent coumarin based HIV PR inhibitors, *Warfarin* and *Phenprocoumon*. Novel isosteres, namely α -(1-hydroxyethyl) and α -acetylcarbonyl have been incorporated with the binding elements present away from the scissile peptide bond site which could lead to better binding interactions with the active site of aspartyl proteases like the HIV PR.

Section II

POLYANILINE SUPPORTED COBALT SALEN CATALYSED SYNTHESIS OF β -PHENYLISOSERINE DERIVATIVES CONTAINING HMC ISOSTERE: REGIO- AND CHEMO-SELECTIVE OPENING OF EPOXIDES FOR THE SYNTHESIS OF MACROCYCLIC PRECURSORS

This section describes the synthesis of library of tripeptides containing the trans-oxirane group for potential irreversible binding with aspartyl protease active site. The transformation is effected by the epoxidation of N-cinnamoyl dipeptides in the presence of *Polyaniline Supported Cobalt(II)Salen* (PASCOS) and 2-methylpropanal in acetonitrile.

generated epoxide of N-cinnamoyl-dipeptides with *meta*-amino phenol, leading to the synthesis of β -phenylisoserine and L-proline containing tripeptide isosteres in the presence of PASCOS, as acyclic precursors for macrolactonisation.



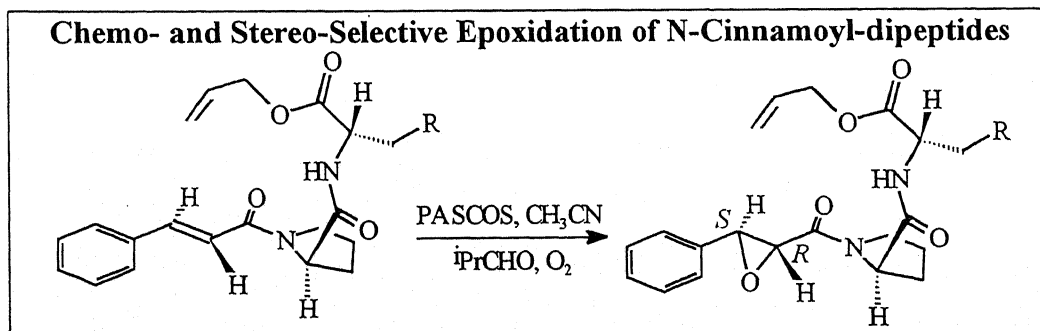
The developed methodology also follows an one-pot epoxidation and its opening by *meta*-amino phenol, of N-cinnamoyl dipeptides in the presence of PASCOS catalyst.

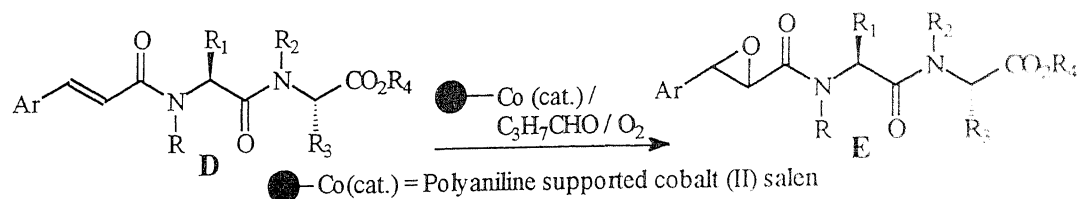
PART B

Section I

CONFORMATIONALLY CONSTRAINED β -PHENYLISOSERINE DERIVED PEPTIDES VIA RING CLOSURE METATHESIS

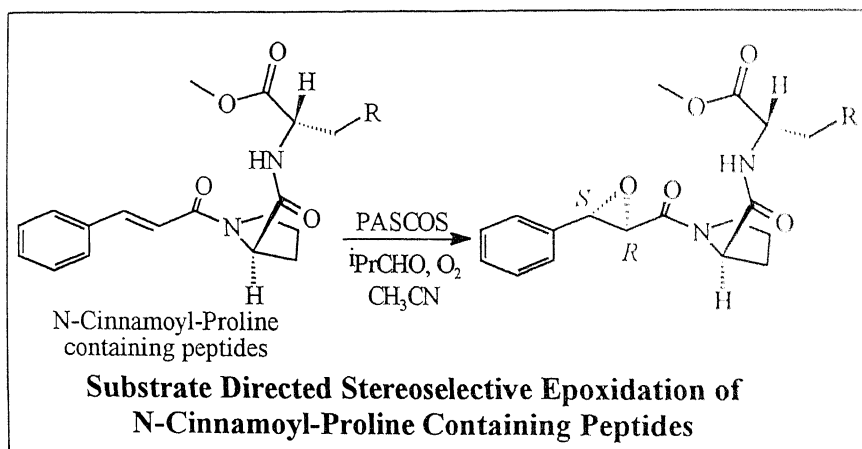
This section describes the synthesis of acyclic precursors containing the β -phenylisoserine derivative, incorporating the α -hydroxymethylenecarbonyl isostere, for cyclisation by Ring Closure Metathesis (RCM), using Grubb's ruthenium benzyldiene catalyst. N-cinnamoyl - mono, -di and -tripeptide allylesters underwent chemo selective epoxidation in the presence of PASCOS catalyst to yield the corresponding glycidyl derivatives. High enantio selectivities were observed for the epoxidation of N-cinnamoyl-proline containing peptides. Opening of the epoxides with N-allyl anisidine in the presence of cobalt (II) chloride led to the synthesis of acyclic diene precursors containing the β -phenylisoserine derivative, for cyclisation via RCM protocol.





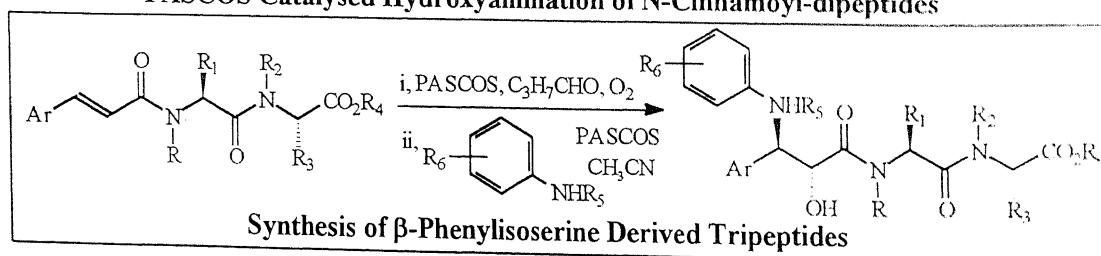
General Methodology for the Synthesis of Small Molecule Tripeptides **E**
Containing Trans Oxirane, From the N-cinnamoyl-dipeptide Systems **D**

It was observed that N-cinnamoyl-proline containing peptides undergo substrate-directed enantiomeric epoxidation, by this protocol. The absolute stereochemistry of the predominant oxirane diastereomer has been assigned on the basis of chemical correlations as (3*S*, 2*R*)-3-phenylglycyl.

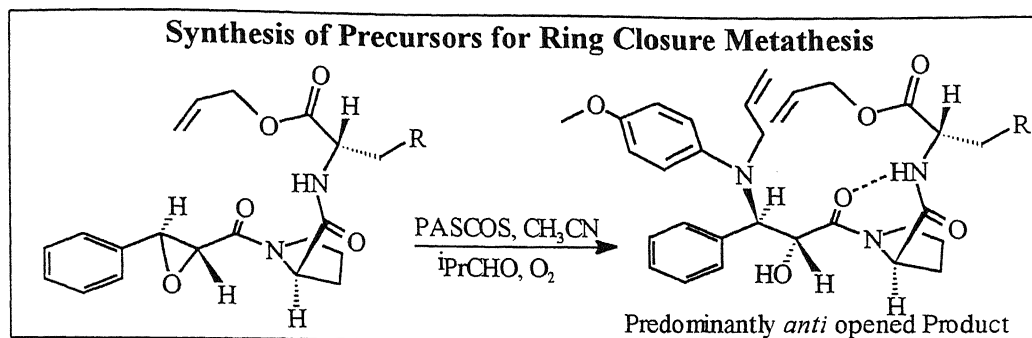


A methodology for the synthesis of library of β -phenylisoserine derived tripeptides has been developed for the hydroxyamination of N-cinnamoyl dipeptides to the corresponding β -phenylisoserine derived tripeptides in one-pot in the presence of PASCOS.

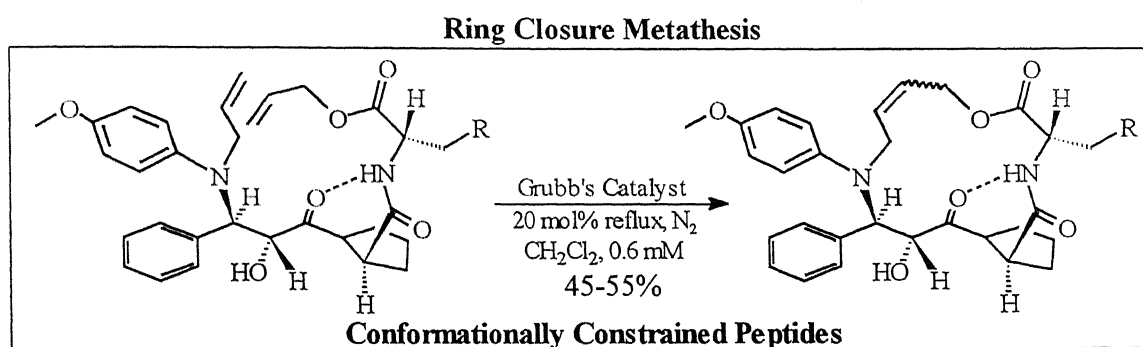
PASCOS Catalysed Hydroxyamination of N-Cinnamoyl-dipeptides



The methodology follows an one-pot epoxidation and its ring opening protocol of N-cinnamoyl dipeptides by various aromatic amines in the presence of **Polyaniline Supported Cobalt (II) Salen** (PASCOS) catalyst. The opening has been observed to proceed predominantly by an S_N2 pathway to yield the *anti* products in major amounts. The section also describes a highly *chemo*- and *regio*- selective ring opening of *in situ*



Cyclisation studies on the diene precursors via RCM using Grubb's ruthenium benzylidene catalyst led to the synthesis of cyclic peptides with proline as the middle residue of the tripeptides. The results of the study emphasise the need for a structural pre-organising element like g-turn in the precursors for cyclisation, which is provided by proline as the *i*=1th residue.



To the best of our knowledge this is the first report of its kind, for the cyclisation of peptides *by directly locking the N- and C- termini of peptidomimics*, via RCM protocol.

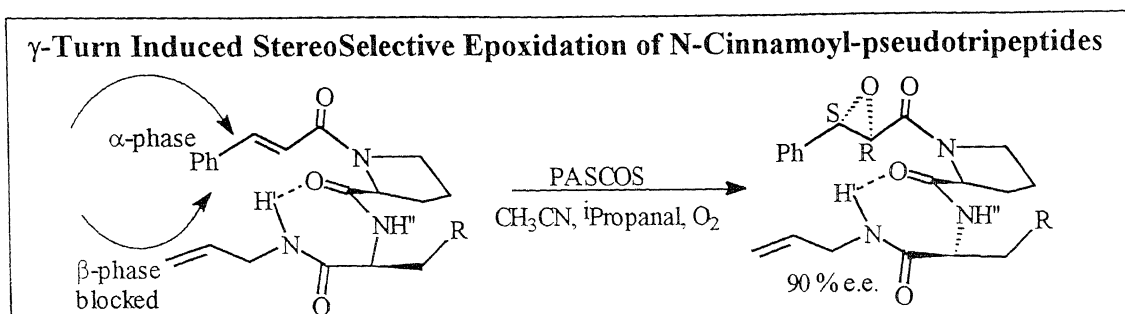
Section II

Peptides as Mimics for the Bio-active Conformation of Phe-Pro Residue of HIV Protease Ligands

This section describes the synthesis of aziridine containing peptides as conformationally constrained phenylalanine derived peptides as potential irreversible inhibitors of HIV PR. Conformational constrain on the Phe has been introduced so that it could lead us towards the bioactive conformation of protease inhibitors. The design is based on the unique specificity shown by HIV PR for the Phe-Pro peptide bond.

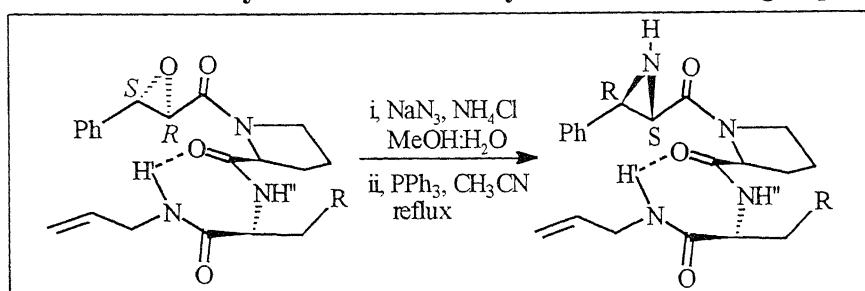
N-cinnamoyl-peptide allylamides undergo substrate directed, highly *chemo*- and *enantio*-selective epoxidation in the presence of PASCOS to lead to the corresponding 3-

phenylglycidic acid derivatives. NMR and molecular model studies suggest the presence of a γ -turn motif, responsible for the observed high facial bias.

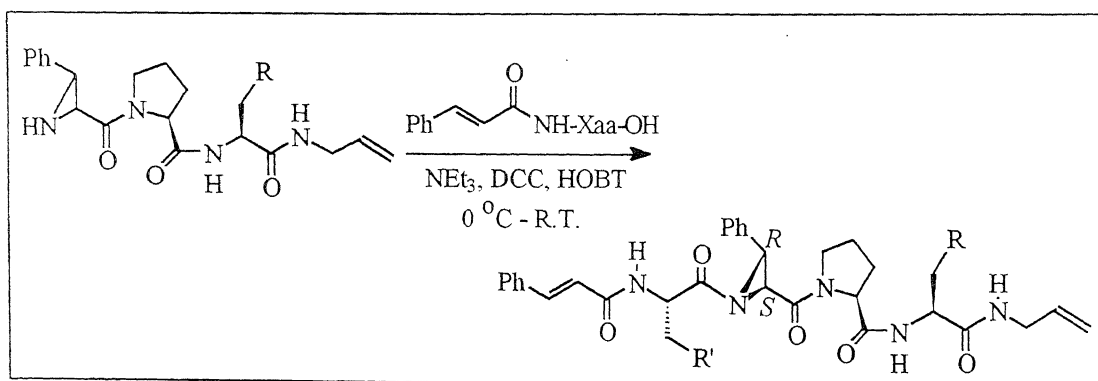


These epoxide containing peptides have been converted to the corresponding aziridine containing peptides via ring opening of the epoxide by azido alcohol and its subsequent ring closure by reaction with triphenylphosphine. These aziridine containing peptides have been proposed to be conformationally constrained Phe-Pro peptidomimics suited for irreversible binding with the active site of aspartyl proteases.

Conformationally Constrained Phenylalanine Containing Peptides



Acylation of the aziridines by cinnamic acid and N-cinnamoyl-amino acids led to the synthesis of oligopeptides incorporating the conformationally constrained Phe analogue.



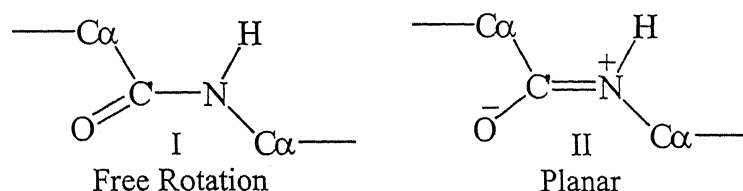
These aziridine containing peptides have been shown to be good synthons for the synthesis of peptides containing a variety of conformationally constrained phenylalanine analogues.

**POLYANILINE SUPPORTED COBALT ACETATE
CATALYSED THREE-COMPONENT COUPLING:
SYNTHESIS OF β -AMINO ACID DERIVATIVES AS
STRUCTURAL ANALOGUES OF HIV PR INHIBITORS**

Introduction to Peptides

Proteins are one of the most fascinating and essential components in the functioning of living systems. Several permutations and combinations of α -amino acids combine to form proteins. α -amino acids are covalently bonded together by an amide bond, called the peptide bond. The semi-rigidity of the amide bond, by virtue of its resonance stabilized structure; the hydrophobicity and hydrophilicity of the side chain groups; inter- and intra-molecular hydrogen bonding interactions between the carbonyls and the amide hydrogens; and the numerous possible types of "turns" that amino acids (each to its extent, by virtue of its unique side chain) introduce in the peptide framework; render the peptide a locally constrained conformational structure called the "Secondary Structure" of the protein. Any understanding and study of proteins requires the understanding to a basic extent, of the different features of these secondary structures.

The secondary structure of each protein is its fingerprint and is primarily based on the sequence of amino acids that form the peptide backbone. Polar side chains of amino acids form hydrogen bonds. The amide groups function as very strong hydrogen donors and the carbonyl groups function as acceptors. Charged and polar side chains fold the poly-peptide such that usually they are at the molecular surface. Proteins are as tightly packed as good molecular crystals. Rotation around the peptide bond in general, is inhibited by resonance (Figure 1).



The two limiting electronic structures of the peptide bond

Figure-1

The stiff peptide bond restricts the poly-peptide chain flexibility appreciably. In addition, the peptide unit is rather bulky and gives rise to substantial steric hindrance¹ which in turn restricts the free rotation around the N-C α and the C α -C' bond of the chain. With a stiff peptide bond and with rather rigid bond lengths and bond angles, the conformation of the poly-peptide chain² is essentially as shown in Figure 2.

The peptide conformation is defined by the ϕ_i and Ψ_i angles (Figure-2). Except for Gly and Pro, all sterically allowed regions for other amino acids are essentially the same. In a hard-sphere model only 8 % - 22 % of the space remains conformationally allowed.³

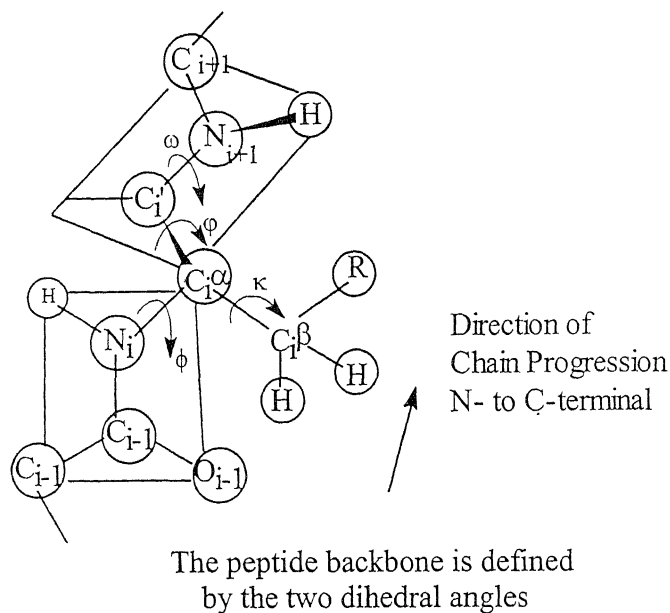


Figure-2

Pro behaves differently because its side chain binds to the peptide nitrogen and fixes ϕ to $-60^\circ \pm 20^\circ$. For Pro, the allowed ϕ -variation reflects different pucker possibilities of the pyrrolidine ring. This results in the reduction in energy gap between *cis*- and *trans*-configuration in proline (~ 3 kcal/mol) making proline as the only natural amino acid that can exist in both the configurations with similar ease.⁴

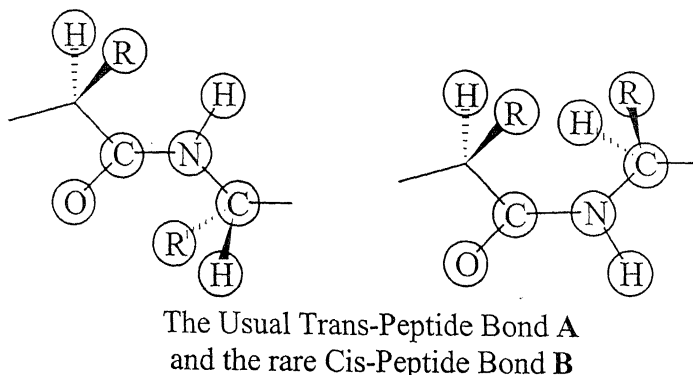


Figure-3

The energy barrier between the *cis* and *trans* structures for linear amino acid containing peptides is ≈ 20 kcal/mol. This is lower only for Pro. Here, concomitant changes in the pyrrolidine ring puckering decreases the barrier. During the folding process in peptides,

Pro residues undergo spontaneous isomerisation since energy difference between the *cis* and *trans* isomers is only about 2 kcal/mol in favor of *trans*. Hence, in comparison, the *trans* bond is superior to the *cis* bond because geometrically it is not as restrictive as the *cis* bond.

Secondary Structures in Proteins

Proteins adopt definite conformational arrangements by unique folding, primarily based on their amino acid sequences. On the basis of covalent structure of its backbone, two of the most common secondary structures in a polypeptide are the α -helix and β -sheet. Whereas α -helix is formed by intra-molecular H-bonding, β -sheets result from the inter-molecular H-bonds between the amide NH and the carbonyl of regularly arranged adjutant poly-peptide chains.

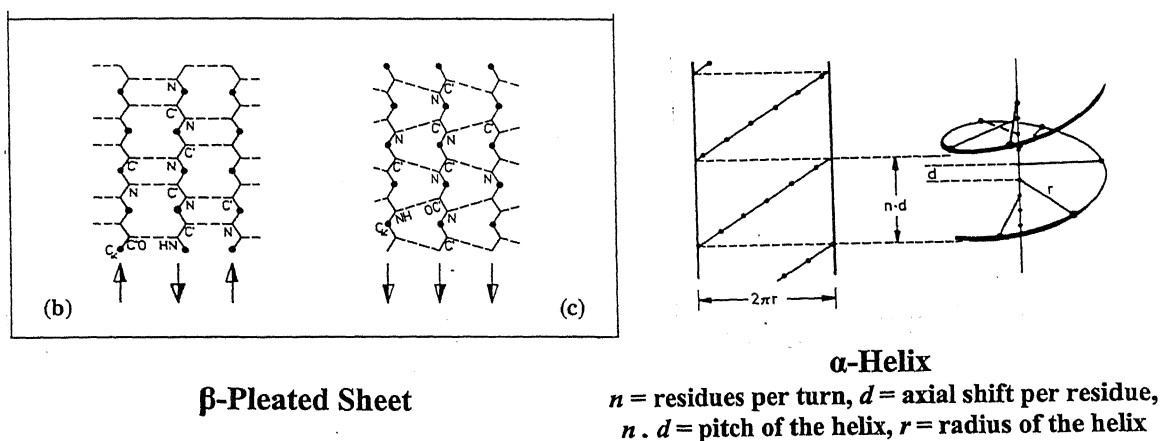
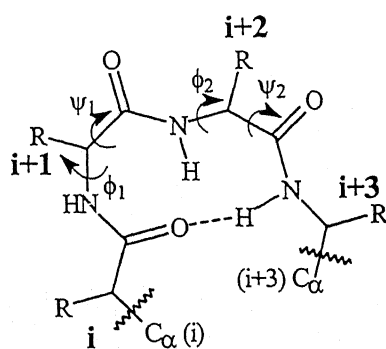


Figure-4

Turns in Peptides



The structure of a β -turn

Figure-5

One important feature that is responsible and essential for the stability of the secondary structures of proteins is the ability of peptide chains to form a sharp reverse turn which

contains a hydrogen bond. Depending upon the number of atoms in the thus formed cycle, through H-bonds, the turns are named as β and γ . There are various types of these turns, all differing in the dihedral angle values ϕ and ψ as shown in Figure-5.

Most of these reverse turns are at protein surfaces and serve as highly specific recognition sites, a feature particularly helpful while designing ligands (e.g. as drugs) for interactions with proteins. These turns are points of least resistance to non-covalent forces trying to bend the chain. There are a large variety of observed reverse turns (Figure-5), demonstrating that none of them has a particularly stable conformation. Any slight changes in the polypeptide chain can either form or break an existing reverse turn. Thus, these turns and twists are as a result of local optimization.

Presence of other elements of secondary structure

In secondary structures linear-group symmetry is found. In all levels above secondary structures, (e.g. β -sheet, which is an aggregation of few secondary structures) point group symmetry is found (e.g. the existence of a C_2 -axis of symmetry in the HIV -1 PR). These have served as primary elements to specifically target drug molecules towards these polypeptides or enzymes. In larger aggregates a space-group symmetry is found. Presence of these elements and a hierarchical order among them, have been instrumental in a better understanding of proteins.

Protein - Ligand Interactions

Proteins are selective in their interactions with cell constituents. In contrast to naturally occurring proteins, chemically synthesized polypeptides of random sequence behave like small children.⁵ Natural proteins were educated by evolution to touch only a small selection of molecules.⁶ This was only possible because they learned to form defined compact structures in contrast to synthetic polypeptides. Specific binding is an individual property of proteins. Consequently, most proteins are specialized in interacting with one or more specific "targets". The smooth physiological operation of living organisms is possible only because a natural protein does not take advantage of the intrinsic capacity of polypeptides to bind all kinds of small molecules, but specialize in a few specific ones.

Enzyme action

Proteins function primarily based on the secondary structures they uniquely adopt, according to the "environment" or the substrate (or the ligand) that they interact with.

Each interaction, in order that the enzyme is specific in its action, takes place only at certain well-defined "fractions" of the proteins, with definite secondary structures, called the "active site" of the enzyme. All differences and therefore all information are restricted to the rather short chains. Proteins are synthesized on ribosomal systems. After synthesis on the ribosomal systems, the polypeptide chain folds spontaneously to an amino acid sequence-dependent globular protein by adopting a state of "lower free energy".⁷ The resulting chain fold determines the "specificity" of the protein.

Metabolism, an essential phenomenon in living systems, is handled predominantly by enzymes. Most prominent among the metabolic activities are ligation with metal ions, neutral molecules, hydrophobic molecules; acting as inter cellular "message" transmitters, etc. Invariably, catabolic activities of enzymes, which are mandatory for the control of excess production of cellular matter, is characterized by the "cleavage" of proteins at the peptide bonds. This phenomenon is called "peptidolysis" or "amidolysis"; and the enzymes that function by cleaving other proteins at the amide bonds are called "Peptidases" or "Proteases". Proteins are cleaved for both the removal of unwanted proteins and the release of required functional proteins from their precursor poly proteins.

Proteases

DNA encodes all the information for the functioning of the cell and hence the living system, in its nucleotide sequence, in the form of "triplet codes". These codes are translated into the essential proteins, with the help of RNA. These proteins thus synthesized from the "DNA factory", usually comprise of many number of proteins, each responsible for different activity, that run serially from one to the other. These proteins, called "Poly Proteins" are further "cleaved" by proteases to release the smaller functional proteins. *An excellent cost effective methodology adopted by the highly evolved nature, considering that the "Translation" process is highly energy consuming.* Proteases in-turn are regulated by enzymes called protease-inhibitors, that act in order to arrest any malfunctioning of the proteases.

Peptidases act by catalytic hydrolysis of specific peptide bonds with a molecule of water, the process being catalyzed by certain amino acid residues, suitably positioned at the active-site of the enzyme. Depending upon the domain of catalytic action on the substrate, proteases are classified into exo- and endo-peptidases. Exo-peptidases act on chain ends and endo-peptidases act on internal amide bonds of the peptide substrate.

Similarly, depending upon the amino acid or the element that catalyses peptidolysis at the enzyme active site, proteases are named and classified into four classes, namely Aspartyl proteases, Cysteine Proteases, Serine proteases and the Metallo proteases.

Protease Substrates

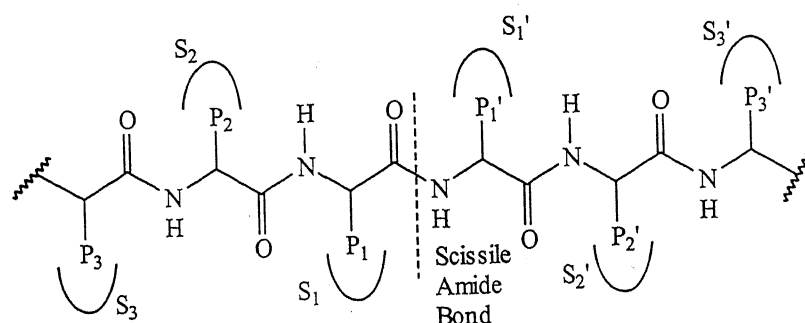


Figure-6

The bond that is cleaved in the substrate by the enzyme is termed as the "scissile" bond. Nomenclature⁸ of enzyme-substrate binding and the process of peptidolysis is as follows. The amino acid residues towards the N-terminal of the substrate, from the scissile bond are designated as P₁, P₂, P₃, etc., and those towards the C-terminal are designated as P₁', P₂', P₃', etc. The corresponding enzyme binding sites are named as S₁, S₂, S₃, etc. and S₁', S₂', S₃', etc. respectively. P_n & P_n' residues of the substrate bind to the enzyme binding sites S_n & S_n', respectively.

Virus

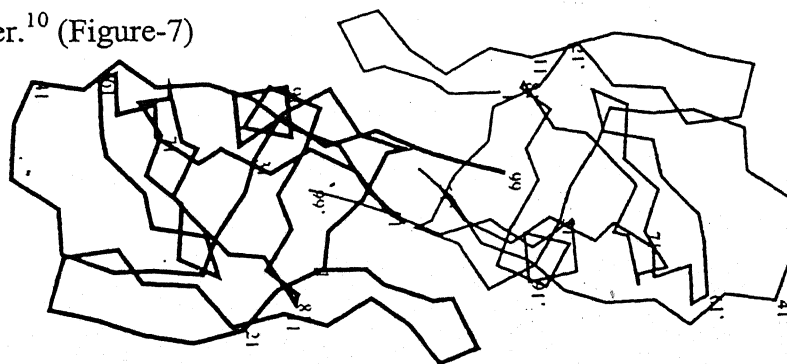
Genetic material for every living organism is stored in the DNA. For the generation of proteins from DNA, there is a double translation mechanism that takes place. First, the DNA is translated into the RNAs, and then to the structural proteins. However, most viri store their genetic information in the RNA. Hence, viri are tougher opponents for target. Once a host is struck, it is easier for the virus to mutate the host DNA and produce the viral proteins encoded by the viral RNA. Viral RNA also encode for the proteins required for their reproduction. The high adaptability of virus makes it a challenging organism to fight against.

Human Immunodeficiency Viral Protease

In the ever growing "*struggle for existence*" among different species, the apparently "recent struggle" seems to be between the arguably most intelligent species, the "homosapiens" and the seemingly equally intelligent viri, the Human Immunodeficiency Virus (HIV). Acquired Immunodeficiency Syndrome (AIDS) is a dangerous, degenerative, disease caused by HIV. The rapid spread of the acquired immunodeficiency syndrome (AIDS) epidemic which causes degenerative traumatic death to its patients has simulated discovery for therapeutic agents to arrest the reproduction and growth of the causative agent, the human immunodeficiency virus (HIV).⁹ Many novel strategies have been tried to combat this growing health threat of global proportions. One such strategy is the inhibition of the viral-encoded protease required for processing of the viral gag and gag-pol poly-proteins.¹⁰

HIV codes for an aspartic protease, known to be essential for retroviral maturation and replication. Kramer et al. suggested that inhibition of this protease, called the HIV-Protease (HIV-PR) would represent an appropriate strategy for interfering with HIV-1 replication in 1986.¹¹ In 1988 it was observed that deletion mutagenesis of the HIV PR gene resulted in the production of non-infectious, immature viral particles.¹² This experiment demonstrated that HIV PR performs an essential function in the life cycle of HIV and thus makes itself an important target for the design of specific antiviral agents for AIDS.¹³

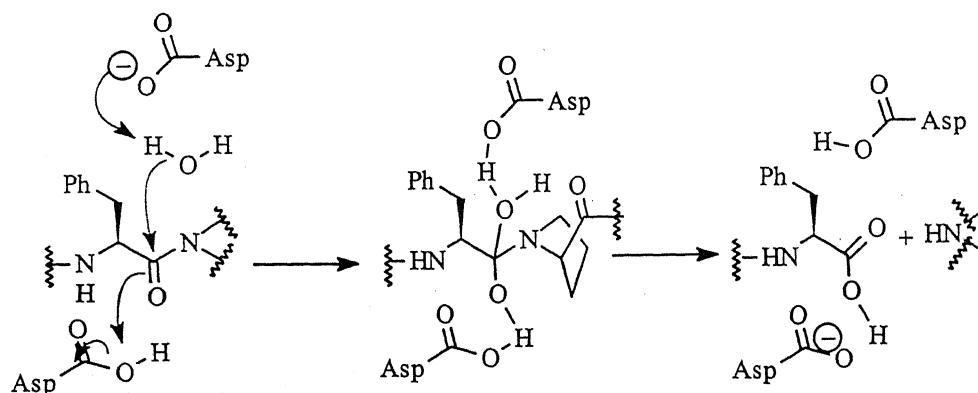
The HIV PR is understood to be an essential factor for processing of the viral gag and gag-pol poly proteins into enzymes and structural proteins necessary for the formation of infectious virions. The protease is a 99 amino acid residue protein that functions as a homodimer.¹⁰ (Figure-7)



A view of the Ca backbone tracing of the HIV Protease dimer, in an orientation emphasizing the interactions in the dimer interface (reproduced from Wlodawer A *et al Science* 1989, 245, 616)

Figure-7

Pepstatin, a proven inhibitor of aspartyl proteases like renin showed inhibition of HIV-PR. This and partial sequence homology led to the discovery that HIV-PR is an aspartyl protease.¹⁴ It is classified as a member of the aspartyl protease family on the basis of its active site sequence similarity and structural analogy to other well characterized, monomer enzymes renin, pepsin and rhizo-pepsin, enethiapsin and penicillopepsin. It is predominantly composed of β -strands. In its active form it functions as a homo-dimer, with a C_2 axis of symmetry in it. Its active site is formed at the interface of the dimer and contains two aspartyl residues, one contributed by each sub-unit.¹⁰



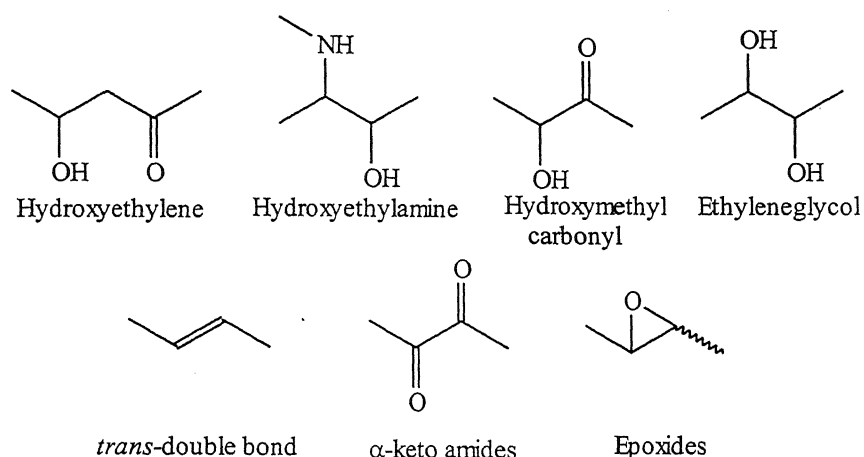
Enzyme reaction mechanism of HIV PR at the Phe-Pro Scissile amide bond :

Figure-8

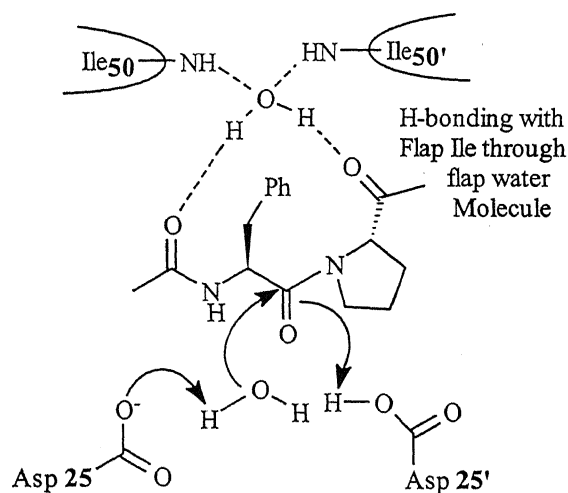
HIV-PR¹⁵ shows high specificity for the selective cleavage of the tyrosine / phenyl alanine - proline amide bond in the matrix capsid domain of the gag-pol poly-protein, a specificity not exhibited by other mammalian cellular proteases which are known to efficiently hydrolyze peptide bonds involving the proline nitrogen. It is this specificity that makes HIV-PR an attractive target for inhibition.¹⁶

HIV-PR Inhibitors

Potent inhibitors of these enzymes can be readily accessed by the incorporation of an isostere that mimics the geometry of the tetrahedral intermediate in place of the scissile bond of the peptide substrate.^{17,18} Much of the work on the design of inhibitors of the HIV-PR has been guided by the crystallographic structure determination of selected inhibitors bound to the enzyme. Generally, these inhibitors are peptide analogues (peptidomimics) designed to mimic a high-energy intermediate (the isosteres) involved during peptidolysis.

**Figure-9**

Various non-hydrolysable moieties, including the aminoacid statine, secondary amines, hydroxyethylene, hydroxyethylamine, ethylene glycol, hydroxymethylcarbonyl, epoxide, trans double bond and ketones (Figure-9) have been incorporated in these peptides to replace the scissile peptide bond.¹⁰

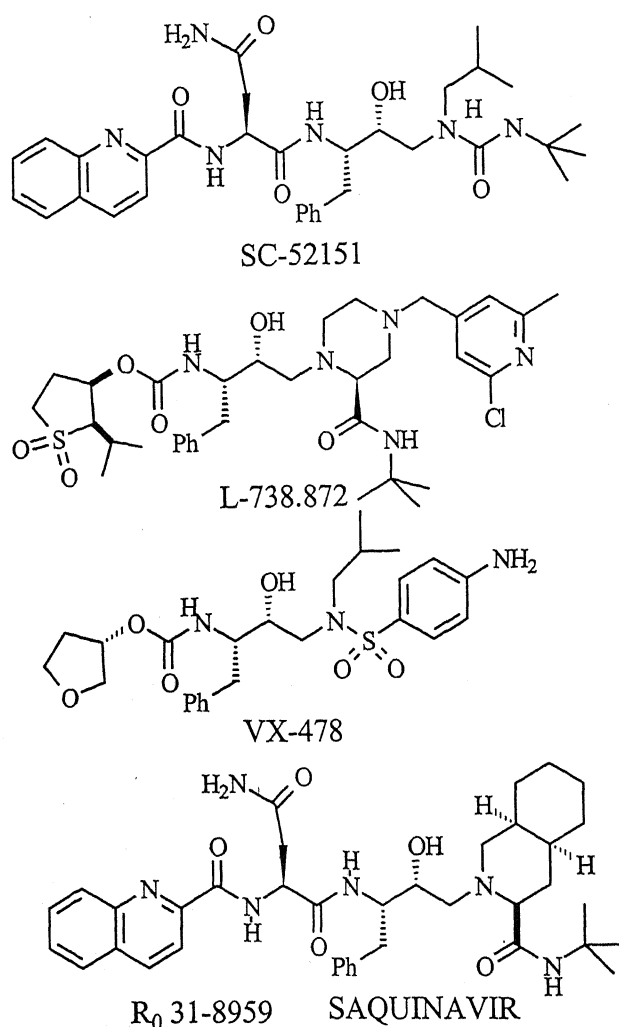


General acid-general base catalysed mechanism of peptidolysis by HIV PR

Figure-10

HIV-PR undergoes considerable conformational changes upon complexation with inhibitors, particularly in the two "flaps" (flexible β -hairpin structures) which move by as much as 7 Å to tightly embrace the ligands. The catalytic mechanism of aspartyl proteases are consistent with a general acid-general base catalyzed mechanism of the two aspartyl residues, with a central water molecule bound between the carboxyl groups of these two residues as the nucleophile (Figure-10).¹⁰

With a wealth of structural information available, HIV-PR has become the most attractive target for computer-aided drug design strategies^{19,20} and this effort has produced a variety of potent inhibitors.²¹⁻³⁹ Saquinavir (Roche), Zalcitabine (Abbott) and Indinavir (Merk) have recently been approved by the FDA and are being used in AIDS therapy in combination with reverse transcriptase inhibitors.



Structures of some of the approved, potent HIV PR inhibitors

Figure-11

However, the ability of the virus to rapidly generate resistant mutants⁴⁰⁻⁴⁸ suggests that there is an ongoing need for new structurally diverse HIV-PR inhibitors with superior potency, pharmacokinetics and efficacy.

Structural mimicry of the native peptide bond Phe/Try- Pro, the bond that is specifically cleaved by the HIV PR, has been aimed to subsequently provide functional mimicry, in binding of the inhibitor to the HIV-PR active site, reversibly or irreversibly. The strategies that exist and are being developed for designing HIV PR inhibitors, invariably

take a leaf or two from the vast studies done for a similar purpose with other aspartyl proteases like the human renin. The utilization of dipeptide isosteres as transition-state mimics in the design of renin inhibitors has been a subject of much interest.⁴⁹ A review of few of the most potent transition state and structural isosteres that have been used in the design of various potent HIV PR inhibitors follows.

Hydroxyethylene isosteres

The hydroxyethylene dipeptide isostere (HDI) transition - state mimetics have been found⁵⁰ to be potent and selective inhibitors of aspartyl proteases such as renin.⁵¹ A feature common to many potential anti-hypertensive agents is the incorporation of hydroxyethylene isostere as a replacement for the scissile Leu-Val peptide bond of angiotensinogen, the substrate that is cleaved by renin.⁵²⁻⁵⁷ The hydroxyethylene isostere **1** and its precursor, amino lactone **2** have been designed and synthesized as potent inhibitors of human renin.⁵⁸⁻⁶²

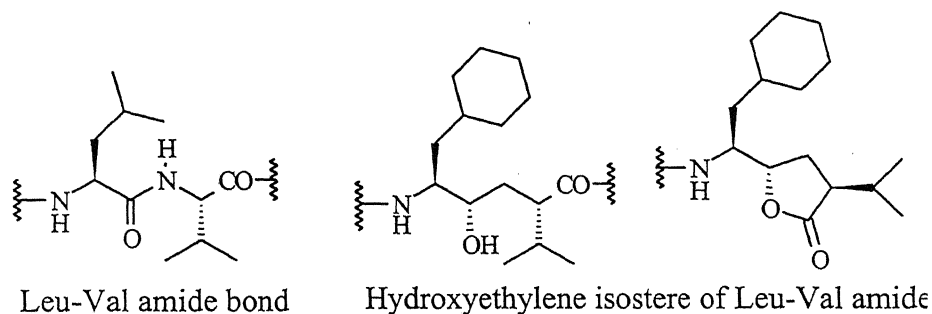
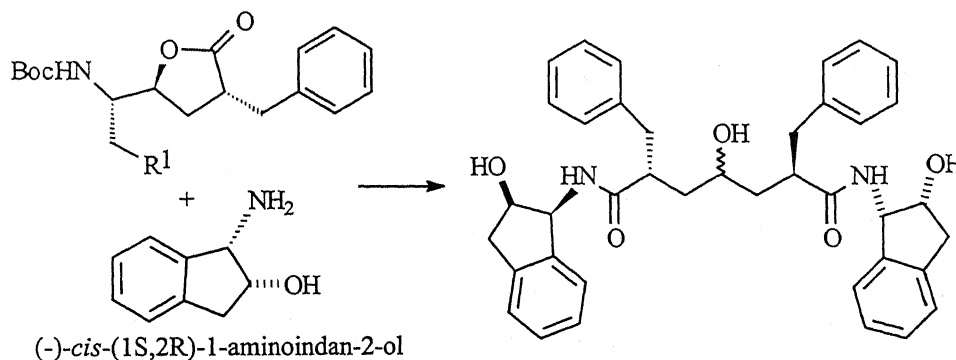


Figure-12



Hydroxy ethylene Dipeptide isostere inhibitors of HIV PR

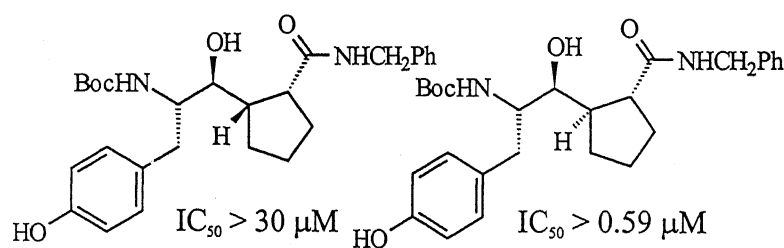
Figure-13

HDIs bearing the cyclic phenylglycine surrogate (-)-*Cis*-(1S,1R)-1-amino-indan-2-ol have been demonstrated⁵¹ to be potent and selective inhibitors of HIV-1 Protease.⁶³ Askin

et al reported⁶⁴ a highly diastereoselective synthetic route for the synthesis of inhibitors containing **7** through the alkylation of chiral α -amino epoxides with chiral amino alcohols (Figure-13).

Although analogues of peptide substrates in which the scissile dipeptide is replaced with mimetics of the proteolysis transition-state (isosteres) are effective inhibitors of purified HIV-1 PR, not all of these inhibitors block viral replication in infected T-cell assays, suggesting that their antiviral activity depends not only on inhibition of the purified enzyme but also on the molecular properties related to their cell permeability and stability.

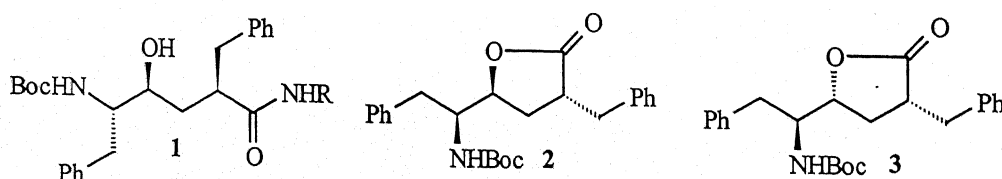
Vara Prasad *et al* have described the stereoselective synthesis of HDI of Leu-Pro.⁶⁵ Similarly, Tyr-Pro HDIs have also been synthesized.⁶⁶ The 3R-diastereomer **10** proved to be more effective inhibitor⁶⁷ of the HIV-1 PR than the corresponding 3S-isomer **11**. Incorporation of the 2S- hydroxy-1R-amino indane as P₂ ligand further enhanced the inhibitory potency. Similar profile is observed with the structure-activity relationship for Phe-HE-Phe based inhibitors of HIV-1 Protease.⁶⁸



Tyr-Pro dipeptide analogues

Figure-14

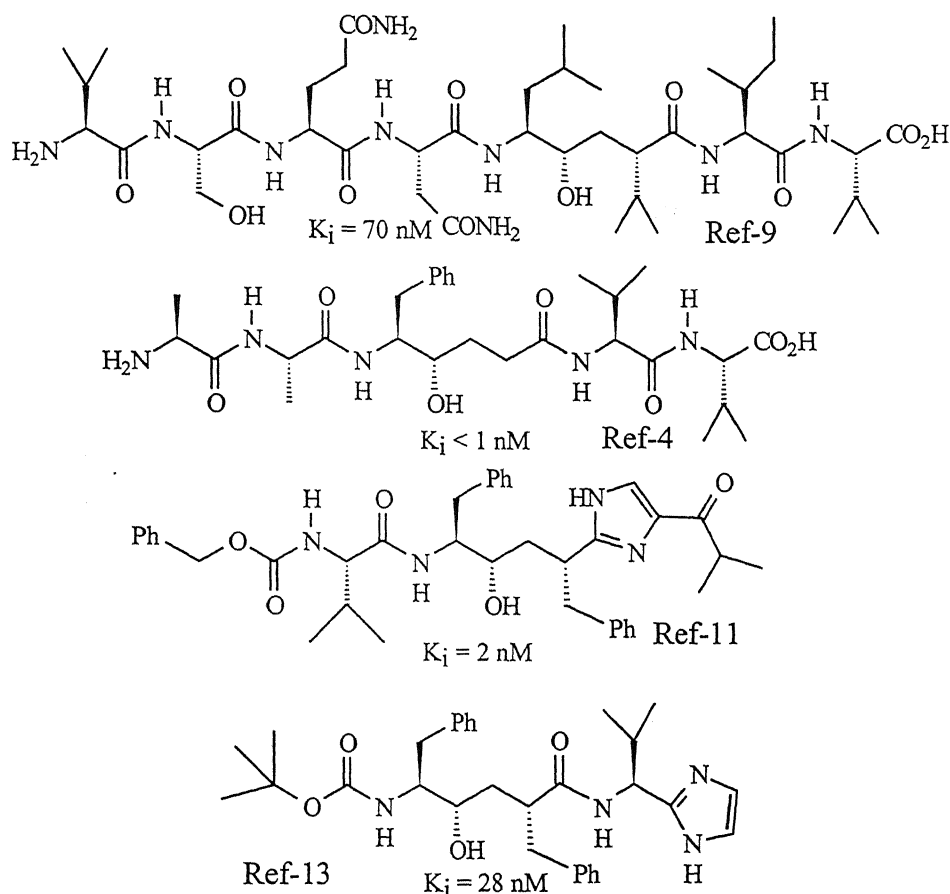
Stephen Hanessian and coworkers reported⁶⁹ an efficient Zinc mediated route for the synthesis of a Phe-Phe hydroxyethylene (HE) isostere utilized for the design of potential inhibitors of renin and HIV-PR. The HE isostere containing Phe-Phe dipeptide analogues were found to be a good tetrahedral transition state mimics for the inhibition of renin and HIV PR.⁵⁰



Phe-Phe Hydroxyethylene isosteres

Figure-15

A few of the other known, potent HIV PR inhibitors containing the hydroxyethylene isostere at the scissile peptide bond are presented below.

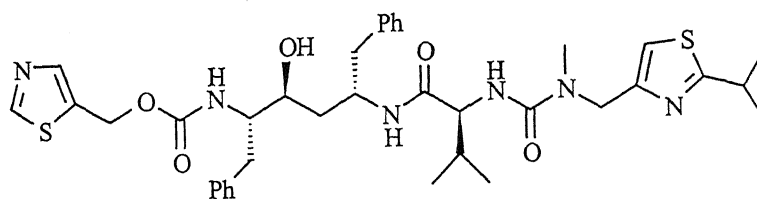


Structures of potent hydroxyethylene isostere containing peptidic inhibitors of HIV PR

K_i = inhibitory constants against HIV PR

Figure-16

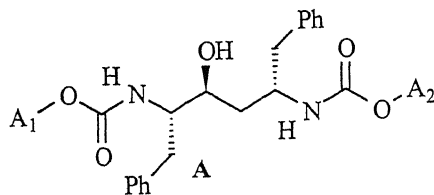
Ghosh *et al* have reported the hexa-hydrofurofuranyl-oxy (HFO) group as a conformationally constrained P2 ligand for HIV PR inhibitors.⁷⁰ X-ray crystal structures of the enzyme bound substrate complexes⁷¹ showed that each of the two ether oxygen atoms of the furofuran, hydrogen bonds to the NH groups of Asp29 and Asp30 respectively, of the viral protease. Following this study, Xiaoqi Chen *et al* reported the study of HFO group as a conformationally constrained P2 ligand for C₂- symmetry based HIV-PR inhibitors containing the Phe-Phe peptidomimic, incorporating the hydroxyethylene isostere core, derived from Ritonavir a licensed, highly potent, HIV PR inhibitor. A number of compounds showed nM level activity against HIV in MT4 cells although showing lower protein binding than ritonavir.



$K_i(78) = 0.7 \text{ nM}$, $IC_{50} = 70 \text{ nM}$

Ritonavir

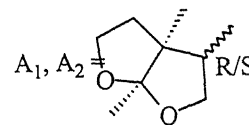
Highly Potent HIV PR Inhibitor



Furofuran containing analogues of A

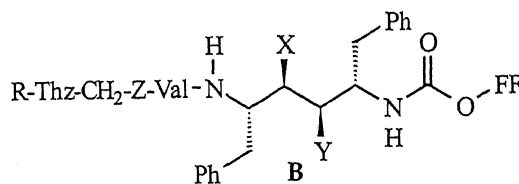
IC_{50} = antiviral activity

$K_i(c)$ = Percentage of inhibition of HIV at concentration C



Hexahydro furofuranyl (FF) analogue

$K_i(53) = 0.5 \text{ nM}$, $IC_{50} = 71 \text{ nM}$



X = H, OH; Y = H, OH; R = H, Me, *i*-Pr; FF = R/S/R,S; Z = O, N

Best inhibitory activity against HIV PR found with B1

B1. X = H; Y = OH; R = *i*-Pr; FF = R; Z = NMe

$K_i(83) = 0.5 \text{ nM}$, $IC_{50} = 10 \text{ nM}$

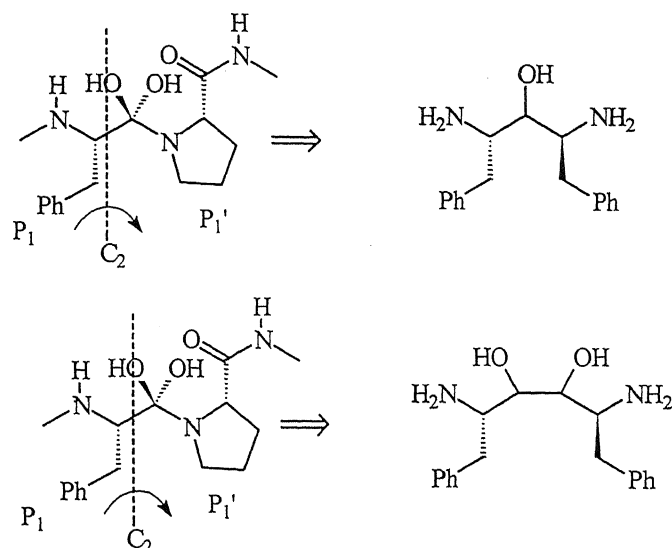
Figure-17

C₂- Symmetric Hydroxyethylamine isosteres

HIV-1 PR, in its active form exists as a homo-dimer with a C₂ axis of symmetry. A wide variety of peptidomimetic inhibitors have been reported based on HIV-PR substrate sequence and three-dimensional structure of the C₂-symmetric, homo-dimeric enzyme active-site.⁷² Symmetric inhibitors are expected to inhibit HIV-PR with greater specificity over their related mammalian aspartic proteases whose substrate binding sites are less symmetric. The design of a C₂ symmetric inhibitor from the tetrahedral intermediate for cleavage of an asymmetric substrate (eg. Phe-Pro), hinges on three factors of operation (Figure-18).⁷³

First, a hypothetical axis of rotational symmetry in the substrate, based on the C₂ axis of the enzyme is chosen; one half of the substrate is arbitrarily deleted. Deletion of P' region

is guided by the greater importance of the P region; then a C_2 operation is performed on the rest of the substrate to generate a symmetric inhibitor.



Design of C_2 Symmetric HIV PR inhibitors

Figure-18

Dale Kempf et al showed that C_2 - symmetric molecules (Compounds **22**, **24**, **25**, **26**) are selective to HIV-1 PR in comparison to human renin.⁷³ X-ray crystallographic studies have also confirmed that **15** make highly symmetric interactions with HIV-1 PR.⁷⁴

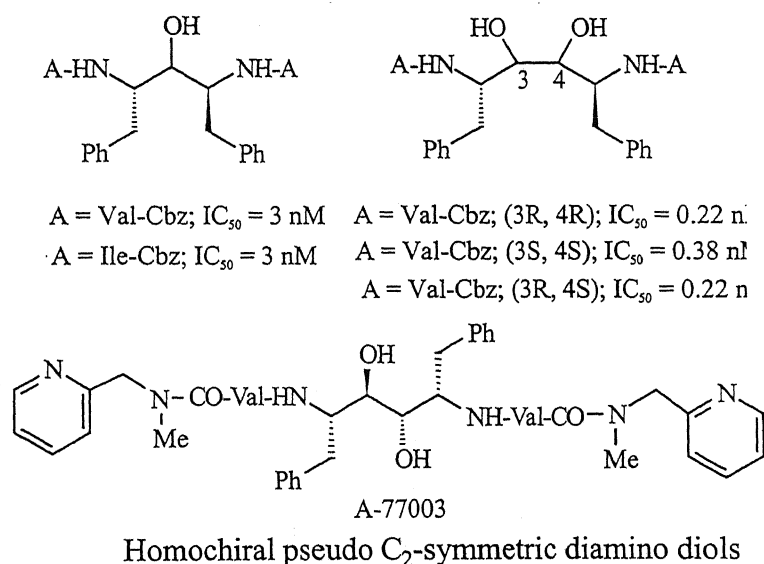
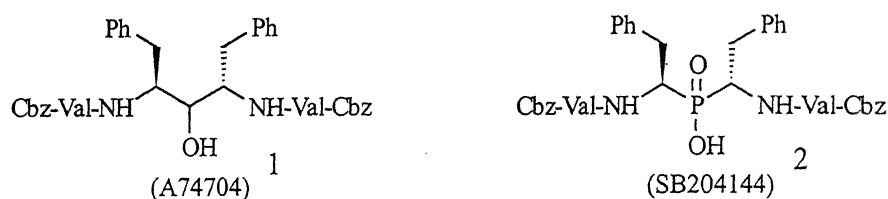


Figure-19

A-77003 (compound **27**) has been reported⁷⁵ by the same group and found to be a highly potent inhibitor of HIV-PR and is already in use in the clinical therapy of AIDS. A-77003 contains the homochiral pseudo C_2 -symmetric diamino diol isostere **21**.

A highly potent, perfectly symmetrical phosphinate inhibitor of HIV-PR, SB 204144, an analogue of its proven hydroxy counterpart (A70704), was synthesized and has been reported to inhibit HIV-1 PR in nanomolar concentration (2.8 nM, pH 6.0).



HIV PR inhibitor 1 and it's equipotent Phosphinate analogue 2

Figure-20

The stereocontrolled synthesis of dibenzylidiamino alcohol **3** and dibenzylidiamino diols **4**, **5**, **6**, core units of symmetry-based inhibitors has been reported⁷⁶ from phenylalanine.

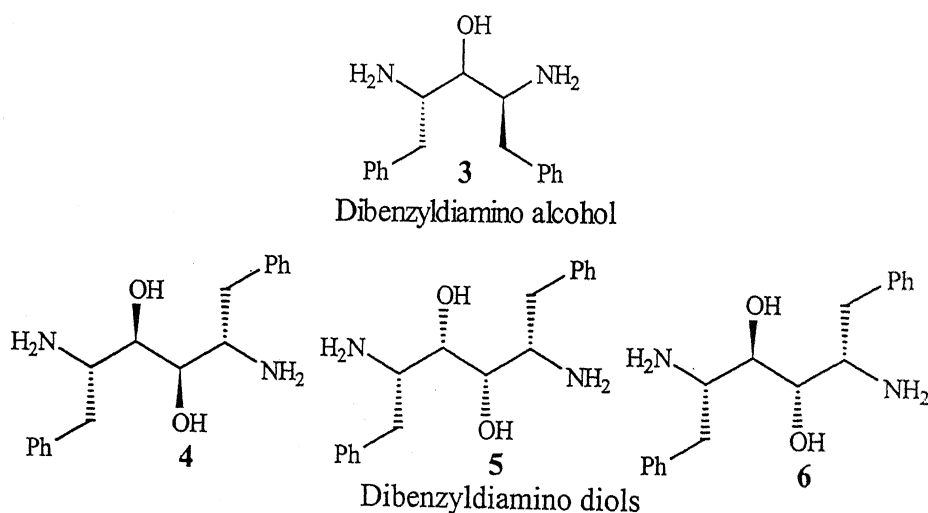


Figure-21

Napthalene Sulphonic acid derivatives

Napthalene sulphonic acid derivatives of C_2 symmetric inhibitors have been reported^{77,78} to be potent HIV-1 PR inhibitors.

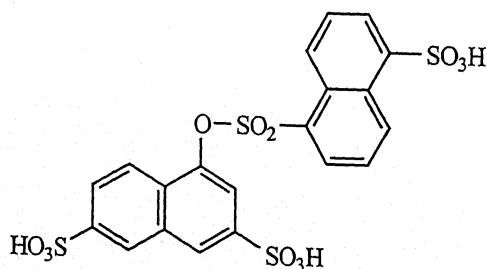
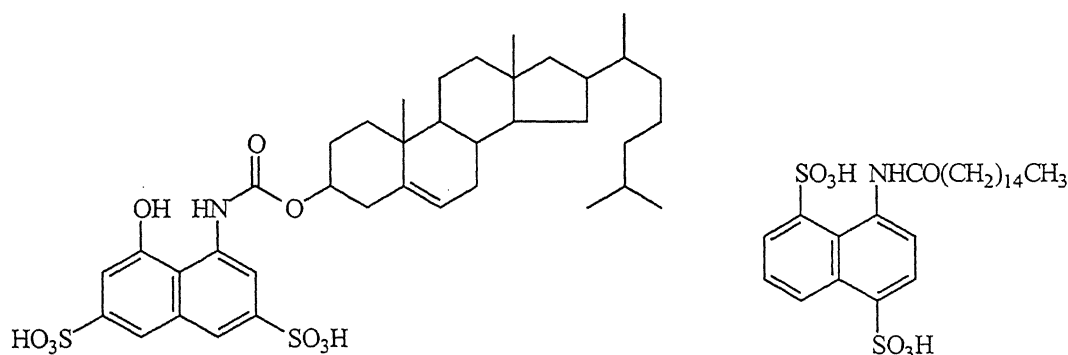


Figure-22

Several naphthalene-sulphonic acid analogues were synthesized using single-step reaction schemes from inexpensive, commercially available starting materials.

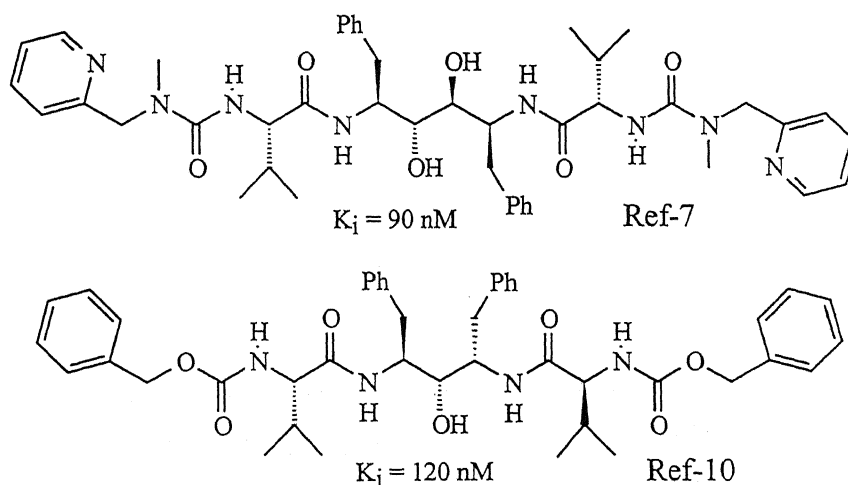
Contrary to thus far observed results, the most promising results were obtained from non-symmetric naphthalene-sulphonic acid derivatives as exemplified by the analogues 32-34 (Figure-22, 23).⁷⁹ The analogues 33 & 34 bearing a cholesteryl and a palmitoyl moiety respectively were equi- and most potent in protease inhibition assay among a library of such molecules.



Structure of naphthalenesulphonic acids with Lipophilic side chains

Figure-23

The structures of a few other potent structure based C_2 symmetric inhibitors of HIV PR are presented below.



Structure of some potent C_2 symmetric and pseudo C_2 symmetric peptidic Inhibitors of HIV PR

Figure-24

Hydroxyethylamine isosteres

Hydroxyethylamine isosteres have been extensively utilized in the synthesis of potent and selective HIV-PR inhibitors,^{70,80-83,87-90} highlighted by the most advanced of them all,

saquinavir,^{84-86,97} which is currently under clinical trial against AIDS. There has been rapid progress in the development of highly optimized P_n and P_{n'} ligands for hydroxyethylamine (HEA) based on HIV-PR inhibitors following the report of saquinavir.⁸⁷⁻⁹³

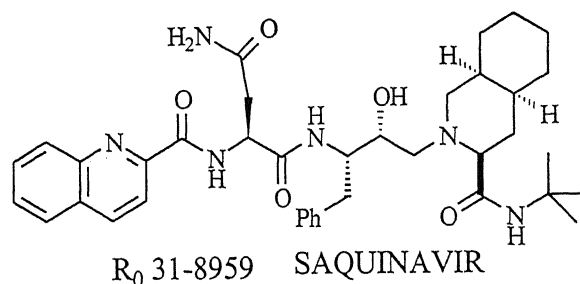
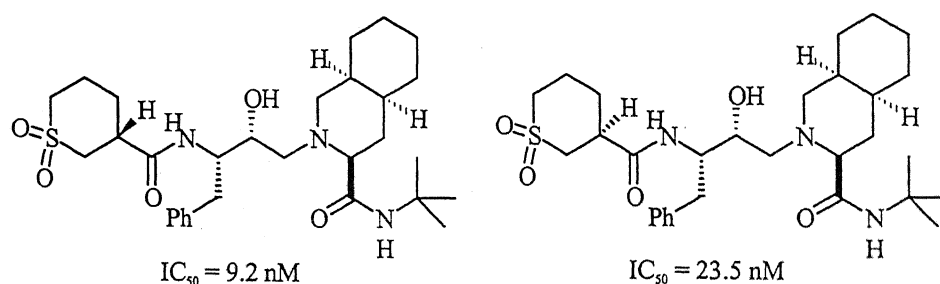


Figure-25

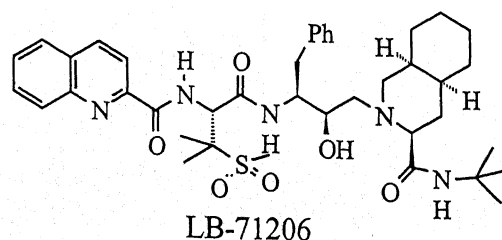
Ghosh and co-workers showed⁹⁴ that cyclic sulfone-3-carboxy amides serve as highly efficient P₂-ligands for R₀ 31-8959 (saquinavir) based HIV-1 PR inhibitors. Incorporation of 3S-tetrahydro-2H-thiopyrancarboxamide-1,1-dioxide into the hydroxyethylamine series resulted in inhibitor **14** with nanomolar inhibitions ($IC_{50} = 9\text{ nM}$). The corresponding 3R epimer **13** showed micromolar inhibitions ($IC_{50} = 2.0\text{ }\mu\text{M}$).

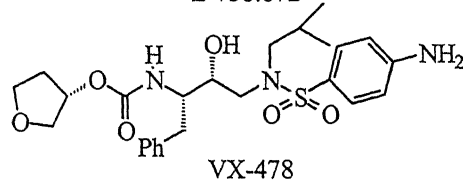
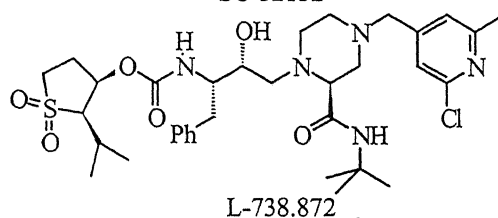
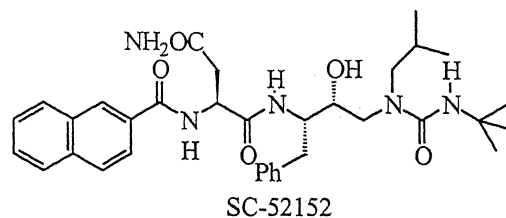
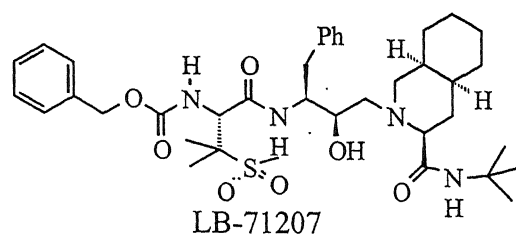


Cyclic Sulfone-3-carboxamide: Novel P₂ Ligand for R₀ 31-8959

Figure-26

A number of structural analogues of saquinavir⁹⁵ (R₀31- 8959) have been designed and synthesized by replacing the P_n P_{n'} side chains.⁹⁷⁻¹⁰¹ A few structural analogues of saquinavir are presented below.



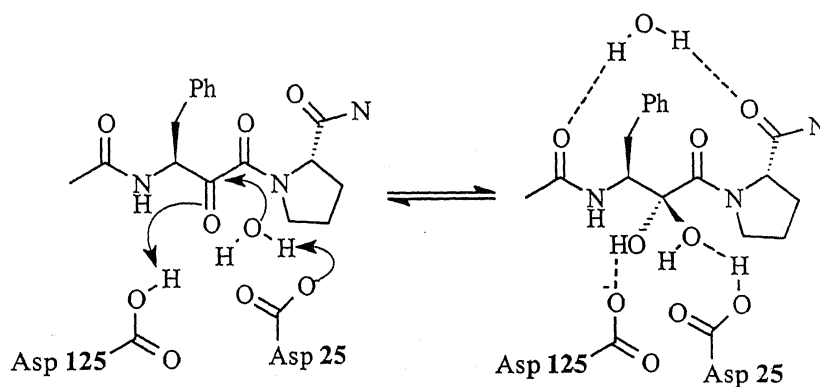


A few structural analogues of Saquinavir

Figure-27

All of these have been reported to have high bio-availability and most of them are currently in clinical trials for the treatment of HIV-PR infection.

α -Ketoamide Isosteres



Schematic representation of general acid-general base mechanism for α -ketoamide inhibitor interaction with HIV PR aspartate group

Figure-28

Activated ketones in general are known to inhibit aspartyl proteases such as renin¹⁰², and serine and cysteine proteases such as α -chemotrypsin and calpain respectively. Ocain and Rich¹⁰³ have shown that ketone moiety of Cbz- Phe- ϕ - Pro-NH β_0 (ϕ = COCONH, instead of CONH), gets hydrated within the active site of HIV-1 PR (Figure-28) and the resulting hydrate is then stabilized through hydrogen bonding interactions with the aspartate residues of the enzyme.

This hydrated form is considered to be a good transition state mimic based on the model presented in figure, supported by X-ray structural studies of this complex.¹⁰⁴ Unlike the case of majority of other inhibitor complexes, the conserved water (WAT-301) of the HIV-1 PR is located rather asymmetric, forming hydrogen bonds between the inhibitor and the two flaps of the enzyme.

Several novel inhibitors, RP1-856, A,B,C & D have been synthesized that showed high potency against HIV-1 PR.¹⁰⁵ These are hexa- and hepta -peptide analogues containing a characteristic dioxoethylene moiety. Here too, the hydrated form of the dioxoethylene moiety seems to mimic the transition state of the substrate bound HIV-PR complex.

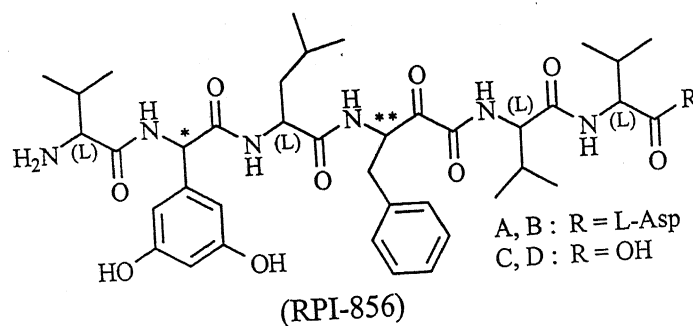
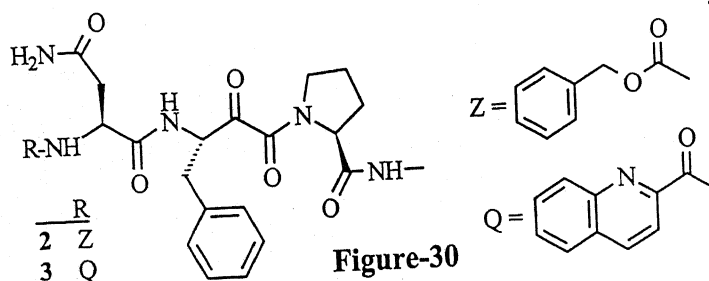


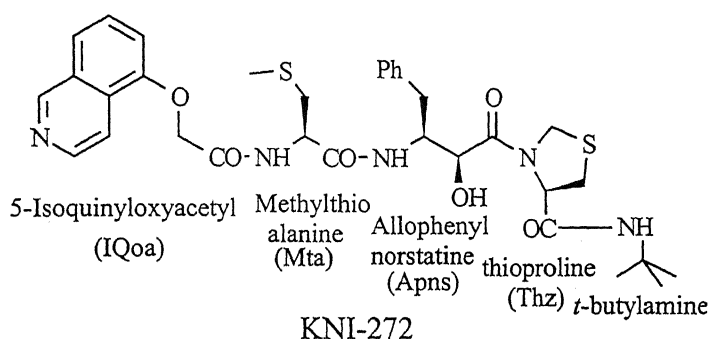
Figure-29

Similarly, smaller tripeptide analogues **44** and **45** have been designed based on the characteristic structure of the analogues of naturally occurring HIV-1 PR inhibitors, namely, RP1-856, A, B, C & D and they showed high inhibitory activity, comparable to that of RP1-856A, against HIV-1 PR in vitro.

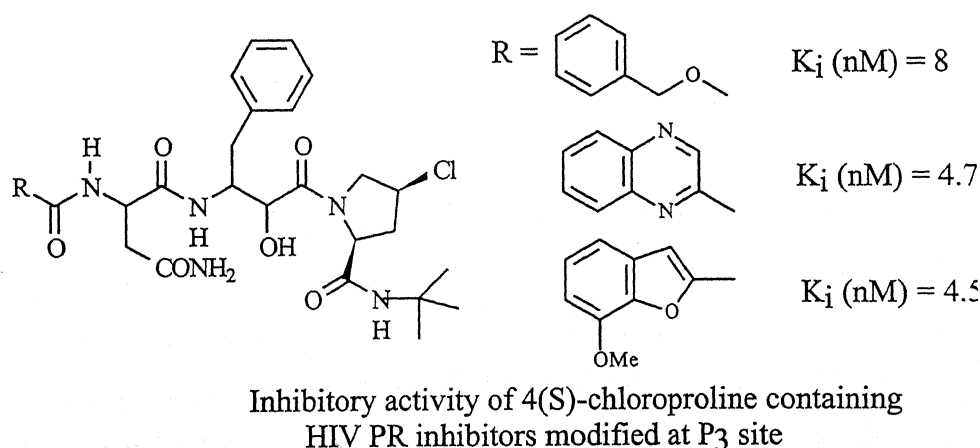


Allophenylnorstatine isosteres

The hydrogen-bond between the aspartyl carboxylic acids at the HIV protease active site and the hydroxyl group of the substrate transition state isostere is very important in the design of tight-binding inhibitors^{106,107}. Based on the transition-state mimic concept, Kiso *et al*¹⁰⁸ designed and synthesized a novel class of substrate-based HIV-PR inhibitors containing an unnatural aminoacid, (2S,3S)-3-amino-2-hydroxy-4-phenylbutyric acid - allophenylnorstatine (Apns/AHPBA), with a hydroxymethylcarbonyl (HMC) isostere at the Phe-Pro scissile site. Lead optimization of substrate based peptides led to the design and synthesis of an inhibitor of picomolar potency, KNI-272.

**Figure-31**

Yuichiro Yabe *et al* incorporated AHPBA at cleavage site of the substrate¹⁰⁹ and after systematic replacement studies of the Pn, Pn' site residues, designed and synthesized tripeptides with very high inhibitory potencies against HIV-PR. They report that the presence of 3-cis-chloro-substituted proline acts as a good P1' replacement and shows high HIV PR inhibitory activity, with various P3 protecting groups.

**Figure-32**

Recently, from a fluorescence-based high-volume broad screening for HIV PR inhibitory activity¹¹⁰ of a set of 5000 dissimilar compounds from Upjohn compound collection

warfarin **48** and phenprocoumon **49** (4-hydroxycoumarins) was identified as a potent inhibitor of HIV-PR replication and spread.¹¹¹

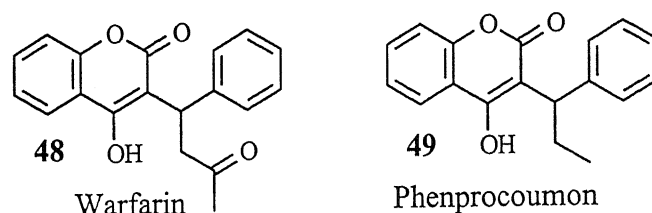


Figure-33

Lead- optimization led to the design of other potent 4-hydroxycoumarins **50**⁹ and **51**¹¹².

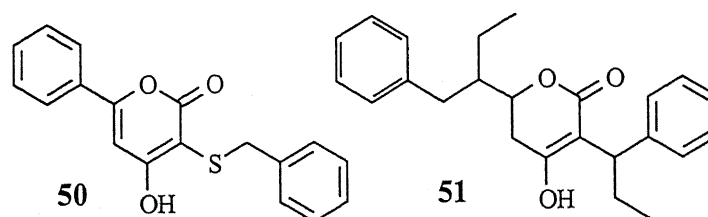


Figure-34

The high binding interactions of the lactone carbonyl moiety in the coumarin moiety with HIV-PR flap isoleucine NHs, suggests that this class of inhibitors represent a useful and novel pharmacophore core for further design of HIV-PR inhibitors.

Lead Optimization

Lead optimization is the series of systematic experiments through conclusions derived from preceding results, conducted to arrive at the “most potent” drug candidates against various diseases.

A historical survey of drug discovery shows that it is possible to improve the pharmacological activity of a “lead” compound by systematic modification of its chemical structure in conjunction with biological and clinical evaluation. Current methods for discovering lead compounds rely both on screening natural and synthetic inventories using a receptor-based or cellular-based assay and on the development of anti-metabolites based on the knowledge of key interactions.¹¹³ From experimental observation at the atomic level of how inhibitors bind to their macromolecule targets, specific interactions that are important in molecular recognition can be inferred. This knowledge can be widely applied to the *de novo* design of novel leads; as well as to the improvement of existing leads. Thanks to the pioneering works of several groups¹¹⁴⁻¹²⁰ it is now practical to introduce an iterative cycle of design, synthesis, evaluation and

crystallographic analysis into the process of discovery and elaboration of lead compounds for pharmaceutical study.

The development of powerful iterative graphic hardware and its associated molecular modelling software has provided a smooth link between the structure of the complex and the ligand design process.¹²¹ These molecular models are “only” an integral part of any effort to develop potent peptide ligands towards their substrates and cannot achieve this goal alone. Hence, it is necessary to perform classical structure

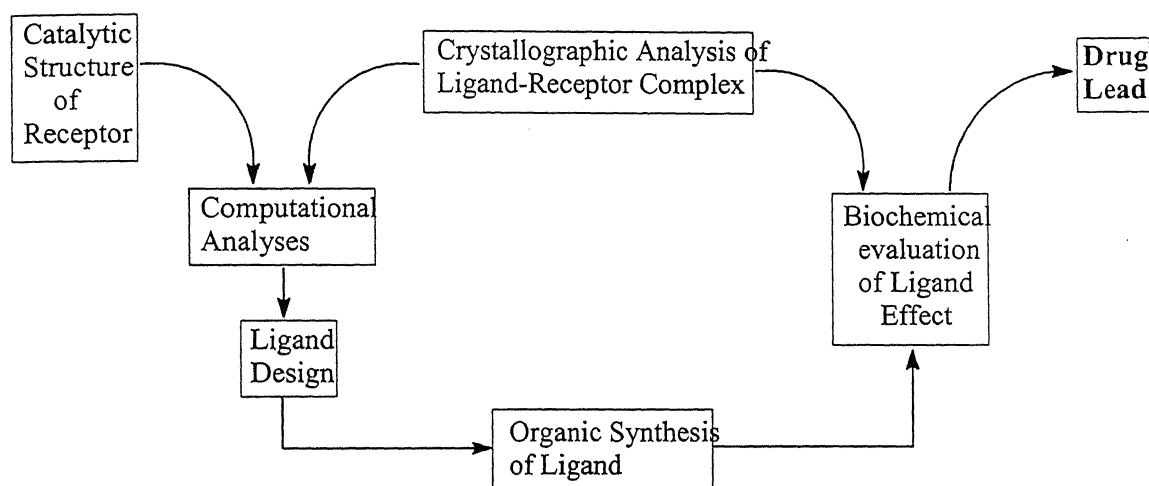


Figure-35

function studies in a systematic way to provide information about the specific aminoacid residues and functional groups in a peptide that are important to biological activity.¹²²

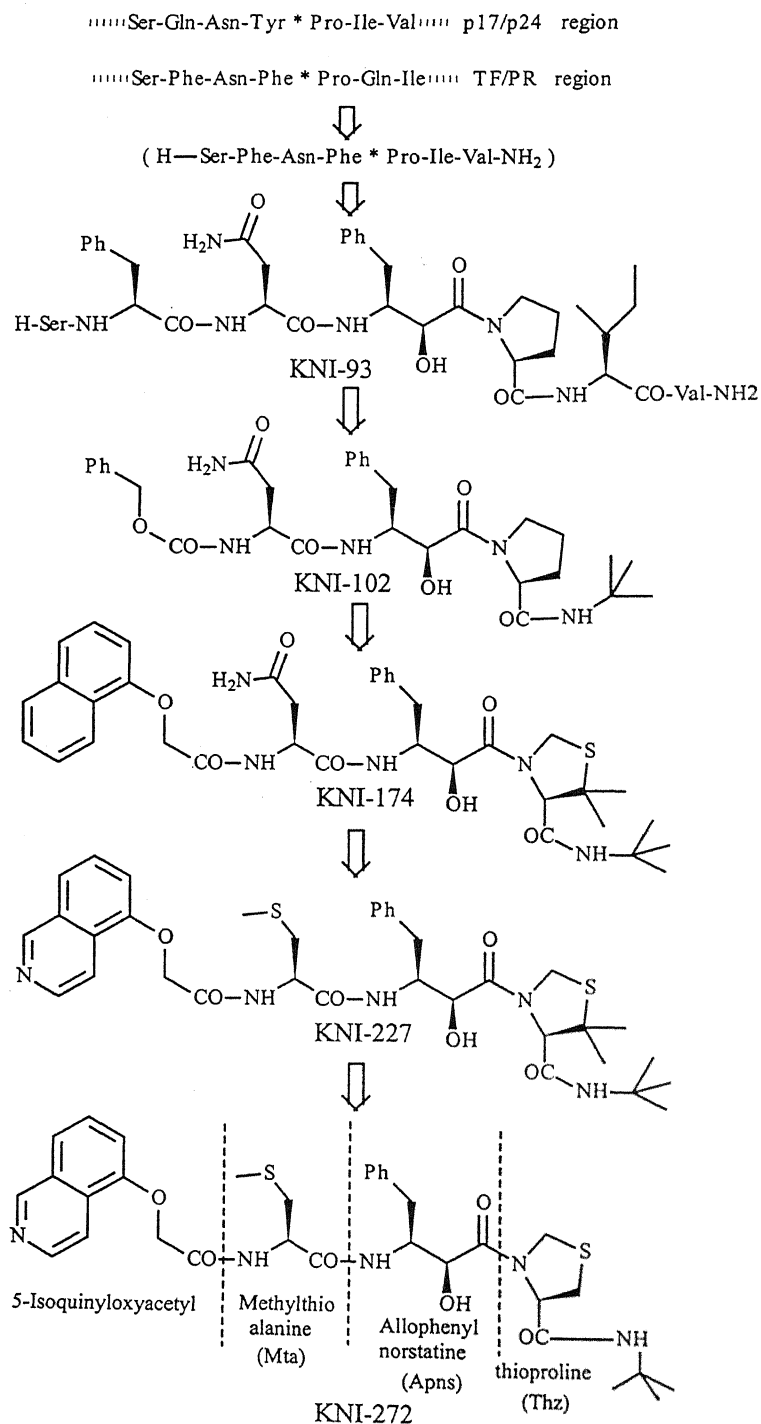
Some of the highly systematic approaches that have been followed, include:

1. Systematic reduction of one aminoacid residue at a time from N and C-termini of the interactive substrate sequence, to determine minimum active sequence and the biologically active sequence.^{123,124}
2. Systematic replacement of peptide bonds with “amide bond replacements” (peptidomimetics-the isosteres).¹²⁵
3. Systematic replacement / manipulation of the aminoacid residues to derive an insight into the difference in the resulting secondary structures (β -strand, α -helix, etc.) and its effect on the stability of the enzyme bound inhibitor complex.
4. Systematic replacement of the side chain moieties with different electronic properties.
5. Systematic cyclization of acyclic peptides and study of the effect of size of the cycle and the side chain moieties.

Presented below are the illustrative case-studies of a couple of such lead-optimized drug candidates as HIV-1 protease inhibitors.

Case study I**Development of kynostatin-KN1-272**

Yoshiaki Kiso *et al* in 1991¹²⁶, reported the design and synthesis of a novel class of substrate-based HIV-PR inhibitors containing an unnatural aminoacid, (2S,3S)-3-amino-



Design of Selective and Potent HIV-PR Inhibitors

Figure-36

2-hydroxy-4-phenyl butyric acid (AHPBA) named allophenyl norstatine (Apns) with a hydroxymethyl carbonyl (HMC) isostere, based on the transition-state mimic concept.^{127,128,129}

Since HIV-PR is unique in cleaving the peptide bond of Phe/Tyr - Pro, unlike other mammalian endopeptidases, the first consideration was to mimic the Phe-Pro scissile site. Hence, the proposed peptidomimic (AHPBA/Apns) was incorporated as a transition state mimic at the P1 site in a heptapeptide Ser-Phe-Asn-Phe-Pro-Ile-Val-NH₂, similar to the TF/PR (please refer to the mnemonic aids in the first page) sequence¹³⁰, at the Phe-Pro scissile site. In a study of varying stereochemistry at hydroxymethylene group of the HMC isostere as part of the peptide, it was found that the (2S) (*syn*) Hydroxymethylene Substituent (HMS) containing inhibitor was more active against HIV PR rather than the (2R) HMS containing isostere, which is found to be active against renin, another known aspartyl protease. Optimization of the chain length led to reduction in size of the heptapeptide to a tripeptide Z-Asn-Apns-Pro-NH-t-Bu (KNI-102). The tripeptide was synthesized and found to be more potent. Here too, the *syn* configuration of the hydroxyl group was preferred. This preference for the *syn* hydroxyl group is exceptional among various HMC-Phe, HE-Pro and HMC-Pro isostere containing inhibitors of aspartic proteases such as HIV-PR, renin and pepsin, which implies the uniqueness of the HMC-Pro structure. The stereochemical preferences of the α -hydroxy groups for different isosteres for better binding with HIV PR and Renin are shown in Table-1.

| Inhibitor type | Preferred geometry of hydroxyl group | |
|---------------------------------------------------------|--------------------------------------|--------|
| | Renin | HIV PR |
| Symmetric Type | | |
| HMC-Phe | Anti | Anti |
| HMC-Pro | Anti | Syn |
| Hydroxy ethyl (HE) (A ₂₄ , 11, 12) HE-Pro | -- | Anti |

Table-1

This discrepancy between the HMC-Pro inhibitors and the hydroxyethyl-Pro inhibitors seem to be due to the conformational difference between the constrained P₁-P₁' peptide

bond of HMC isosteres in contrast to the relatively flexible methyleneamine bond of the HE isostere.

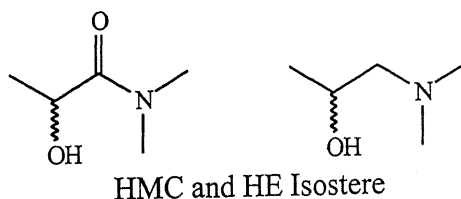


Figure-37

Thus, the critical syn hydroxyl group interacted with the aspartic acid carboxyl groups of HIV-PR active site as a transition-state mimic.¹²⁹ Lead optimisation of the tripeptide KN1-102 led to the replacement of the pyrrolidine of P₁' site with thiazoline or dimethyl thiazoline, resulting in higher potency (KN1-174; K_i = 6.8 pM). From NMR, X-ray and molecular-modelling studies¹³¹⁻¹³² it was concluded that the bioactive conformation of proline in KN1 compounds was *trans* and hence this constrained conformation might be responsible for the high activities.

Considering the subtle balance in lipophilicity and molecular size, the 5-isoquinolinoloxycetyl (IQoa) moiety was incorporated at the P₃ position along with the preferred side chain. Thus resulted the superactive HIV-PR inhibitors, kynostatin (KNI-227; K_i = 2.3pM) and Kynostatin (KN1-272; K_i = 5.5 pM)¹³⁵⁻¹³⁸ (Figure-36).

Case Study-II

Rational Design of Peptide Based HIV-Protease Inhibitor

Noel A.Roberts et al, in 1990 reported¹³⁹ their rational design of a transition-state mimetic peptide-based inhibitor of HIV PR that was seen to have nanomolar inhibitory potencies. Among various transition-state mimetics *viz a viz* hydroxyethylene isosteres; phosphinic acid; reduced amide; statine types and hydroxyethylamine mimetics; the hydroxyethylamine isostere was chosen since it most readily accommodated the Phe-Pro and Tyr-Pro dipeptides, characteristic of substrates that are cleaved by retroviral protease HIV-PR, in it.

Enzyme binding analysis of compounds based on the *pol* fragment Leu165-Ile169 containing the transition state moiety (HEA)-Phe and (CH(OH)CH₂N)-Pro in place of the Phe-Pro scissile bond, indicated that for binding, the stereochemistry best suited at the

hydroxyl-carbon of HEA was R and the compound **2** had the minimum & optimum size required for potent inhibition.

| S.No | Stereo-Chemistry at -CHOH | Inhibitor structure | IC ₅₀ (nM) |
|------|---------------------------|--------------------------------------------------------------|-----------------------|
| 1 | R | Z.Pheφ[CH(OH)CH ₂ N]Pro.O ^t Bu | 6500 |
| 2 | R | Z.Asn.Pheφ[CH(OH)CH ₂ N]Pro.O ^t Bu | 140 |
| 3 | R | Z.Leu.Asn.Pheφ[CH(OH)CH ₂ N]Pro.O ^t Bu | 600 |
| 4 | R | Z.Asn.Pheφ[CH(OH)CH ₂ N]Pro.NH ^t Bu | 210 |
| 5 | R | QC.Asn.Pheφ[CH(OH)CH ₂ N]Pro.NH ^t Bu | 23 |
| 6 | R | Z.Asn.Pheφ[CH(OH)CH ₂ N]PIC.NH ^t Bu | 18 |
| 7 | R | Z.CNA.Pheφ[CH(OH)CH ₂ N]PIC.NH ^t Bu | 23 |
| 8 | R | QC.Asn.Pheφ[CH(OH)CH ₂ N]PIC.NH ^t Bu | 2 |
| 9 | S | QC.Asn.Pheφ[CH(OH)CH ₂ N]PIC.NH ^t Bu | 470 |
| 10 | R | QC.SMC.Pheφ[CH(OH)CH ₂ N]PIC.NH ^t Bu | 12 |
| 11 | R | QC.Asn.Pheφ[CH(OH)CH ₂ N]DIQ.NH ^t Bu | <0.4 |

Z = benzyloxycarbonyl; Pheφ[CH(OH)CH₂N]Pro indicates the replacement of the imide group (CON<) in the Phe-Pro peptide bond by the hydroxyethylamine (HEA) moiety; QC = quinoline-2-carbonyl; PIC = piperidine-(2S)-carbonyl; CN = β-cyanoalanyl; SMC = S-methyl cysteinyl; DIQ = (4aS,8aS)-decahydro-3(S)-isoquinolinecarbonyl.

Table-2

The further structural requirements of **2** for optimal binding to HIV-1 PR was done by synthesizing more than 100 compounds in which the steric and electronic properties of each side chain and terminal substituent were individually modified. High potency of **5** & **8** indicated the presence of large P₃ hydrophobic pocket. Asparginyl group best suited the P₂ site. Similarly benzyl group best suited the P₁ site. However, varying the pyrrolidine ring with piperidine-2-(s)carbonyl (**6**) and (S,S,S)-decahydroisoquinoline-3-carbonyl (**11**) and incorporating combinations of preferred side chains into individual molecules resulted in the generation of several very potent inhibitors of HIV-1 protease and ultimately to Saquinavir (**11**), one of the most potent of inhibitors known today and already in its clinical trial against AIDS in HIV infected patients.

Need for Large Number of Small Molecular Libraries

Enzymes are the prime factors of biological activity in physiological systems. They function by interacting with intra / extra cellular molecules (ions, oligopeptides, etc.), thereby undergoing certain secondary structural changes, resulting in biological

processes. To understand and "manipulate" such processes, manipulation of the substrates, by synthesizing iso-structural molecules that interact with the active site of the enzyme is essential. In most biological processes, these substrates are macromolecules. Hence these manipulations require very efficient and fast methodologies, for the synthesis of even a small number of molecules each, incorporating one of these manipulations. Thankfully, it is by now understood that it is only at very selected sites called the "active-site", of the enzyme that these biological processes get triggered. Similar is the case with the substrate, again thanks to the specificity shown in the interaction with their substrates by enzymes. This has eased the manipulation routine by cutting down the length of substrate-mimicing inhibitor macromolecules to that part of the substrate that "meaningfully interacts" with the enzyme. It is also easier to systematically introduce greater steric, hydrophobic / hydrophilic, electronic and other functional diversities in small molecules. The ease of their synthesis is an immediate corollary.

The most common and effective manipulation technique in the case of inhibiting peptidases is the incorporation of an isostere that mimics the geometry of the (peptide hydrolysis) tetrahedral intermediate in place of the scissile bond of the peptide substrate.^{17,18} There are various techniques to address the need for manipulations, for each kind of enzyme-substrate interactions. However, unfortunately these molecules are not all bioactive and fail as drug candidates due to poor oral availability, short circulating half-lives and metabolic instability.¹⁴⁰ The reasons for this vary, in terms of large size of the molecules, their peptidic nature and imbalance from the optimal hydrophobicity / hydrophilicity of the molecules which has a direct effect on its transport to the target site. In order to generate therapeutically useful leads, the compounds must have both high affinity and favourable pharmacokinetic properties. The combination of these two requirements renders an *a priori* design of lead structure based on the peptide substrate very challenging. Therefore, the identification of potent and bioavailable leads has required the time consuming synthesis and evaluation of a large number of different non-peptidic compounds (non-peptidic for better metabolic stability). The availability of efficient and rapid techniques for mass screening such molecules also demand equally efficient routes to supply these molecules.

With the advances in computer-aided molecular design (CAMD) studies, drug chemistry has taken a new route towards finding leads for drugs against some existing "known"

diseases (e.g. AIDS). However, CAMD relies on and is 'only' complimentary to the X-ray structural data that are available for few of the complexes that are formed between the target enzyme and the substrate. CAMD utilizes the data of thousands of molecules to identify hypothetical drug candidates. Two such examples for the development of potent inhibitors through CAMD are the design of cyclic ureas¹⁴¹ and warfarin^{9,112,142} as leads against HIV PR.

These factors have thus demanded, in recent time efficient routes to the synthesis of small molecules in large numbers.

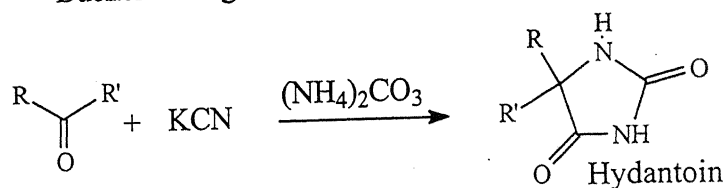
Meeting Demands with the Required Supplies

Solid -phase peptide synthesis, introduced by Merrifield¹⁴³ has led to the rapid synthesis of diverse oligopeptides. Ease in isolation and “greenness” of the reaction conditions in solid phase syntheses are obviously seen to be positive factors for the synthesis of large number of molecules. However, this essentially needs reactions that can build the required small molecules in single or very few steps / transformations. The strength in polymer supported substrate / reagent synthesis would be re-doubled by methodologies that can combine in one-pot, three or more individual components to arrive at one molecule containing all these individual components, covalently bound together. This also results in "diversity" in the synthesized small molecules, a golden-requirement for optimization of lead molecules to be used as therapeutically useful drug candidates. Such a methodology by which three or more individual components covalently combine together in one pot to give new molecules containing all the individual components is called the “Multi Component Coupling” Procedure (MCC).

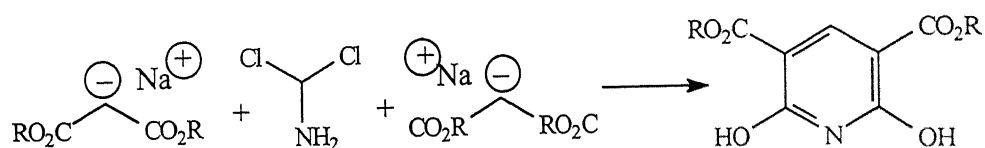
MCC procedures have assumed greater significance, in the realm of developing "Combinatorial synthesis" to greater advantages e.g. for drug synthesis and screening. Combinatorial synthesis although it comprises of such diverse techniques today, that nearly every group involved in it has its own interpretation or variation of the technique,^{144,145} is defined as the methodology of molecular tailoring through which a large number of structurally diverse molecules may be synthesized in a time and resource effective manner. In the context of the discussions in the following studies it is worthy to realize the importance of lead-optimization during drug synthesis and hence the need for efficient MCC procedures. Thus, a methodology like MCC is just “what the doctor ordered for”, for the rapid synthesis of numerous small molecules with a possibility of

diversity at the Pn, Pn' sites. A number of such coupling procedures are known to date. A few of them are listed below.

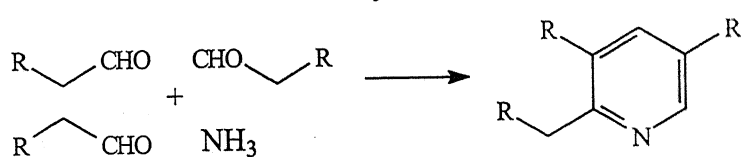
Bucherer Bergs Reaction for synthesis of Hydantoins



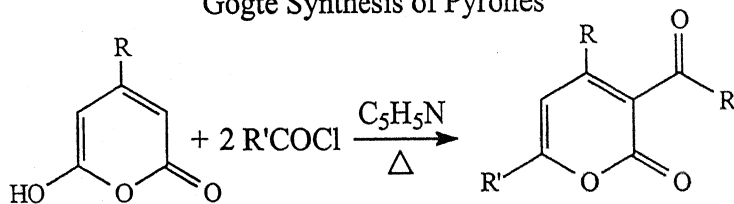
Gattermann-Skita Reaction for the synthesis of Pyridines



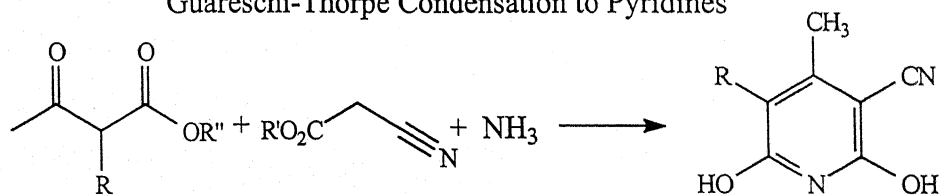
Chichibabin Pyridine synthesis



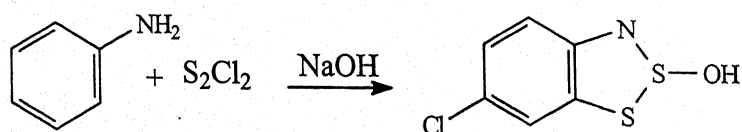
Gogte Synthesis of Pyrones

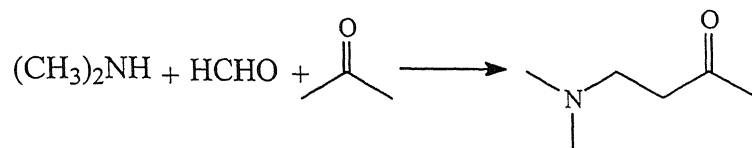


Guareschi-Thorpe Condensation to Pyridines

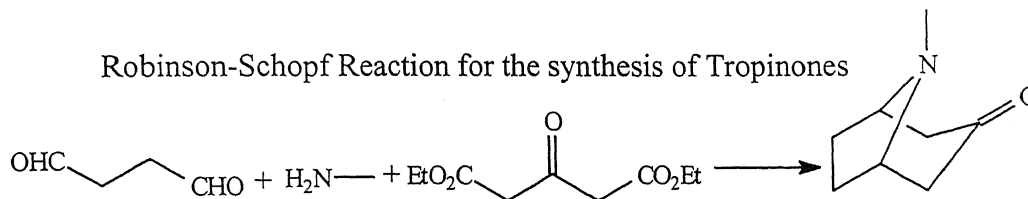


Herz Reaction for the Synthesis of Thiazothiazonium halid

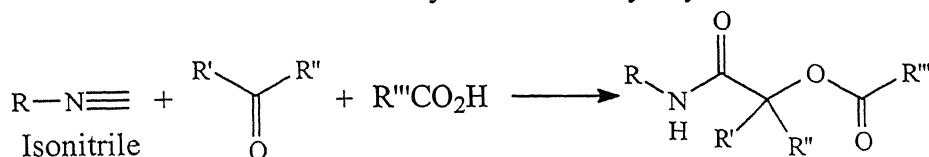


Mannich Reaction for the synthesis of β -amino carbonyl compo

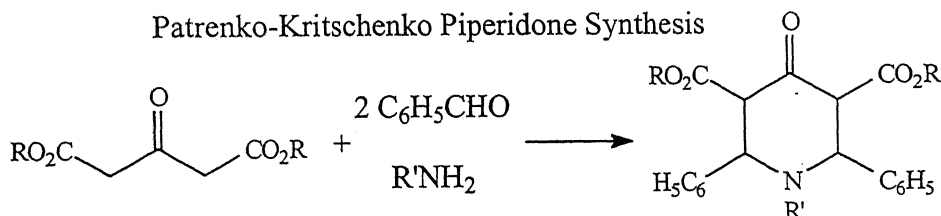
Robinson-Schopf Reaction for the synthesis of Tropinones



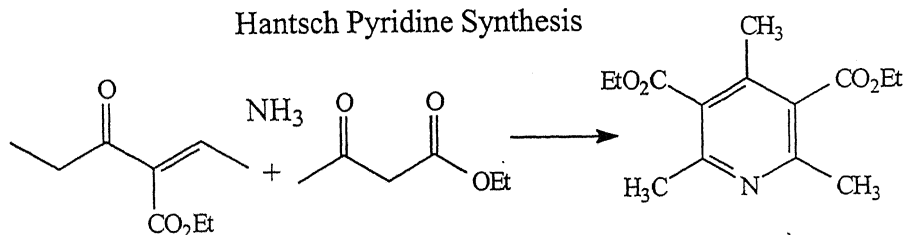
Passerini Reaction for the synthesis of 2-acyloxy amides



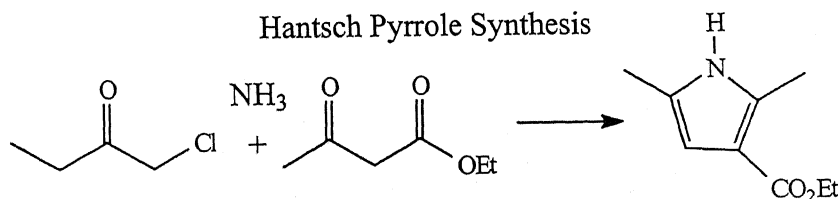
Patrenko-Kritschenko Piperidone Synthesis



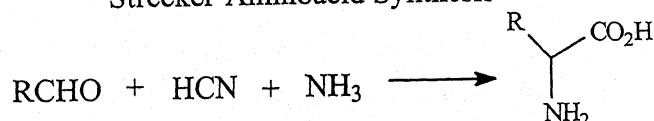
Hantsch Pyridine Synthesis

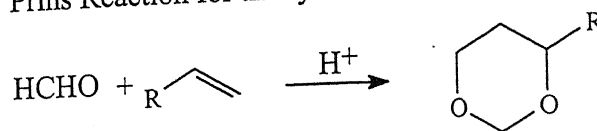


Hantsch Pyrrole Synthesis

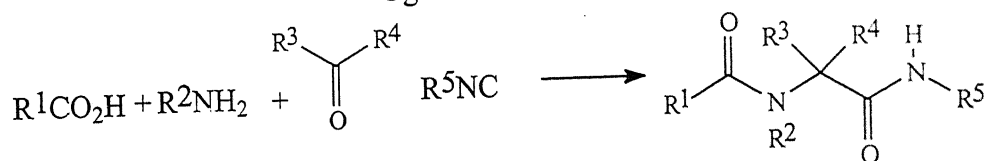


Strecker-Aminoacid Synthesis



Prins Reaction for the synthesis of *meta*-dioxane:

Ugi Reaction



Pauson-Khand Reaction

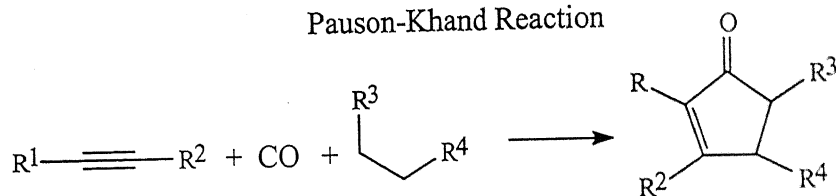


Figure-38

Polymer Supported Catalysts

While solid-phase peptide synthesis introduced by Merrifield has in part successfully dealt with the problem of the need for synthetic methodologies to prepare large number of molecules in a time efficient manner, it falls short in its disability to synthesize gram or even milligram quantities of these molecules. It also requires that the starting material be initially suitably functionalised. Nevertheless, it has provided a good lead. Instead of supporting the *substrate*, it would be more economical and suits our requirements to support the *reagents* on the polymer bed.

Thus, the range and scale of such reactions would then be as many as the number of reagents that can be suitably tailored on polymers. This concept is even more attractive if the supported reagent acts catalytically. Transition metals are known to catalyze a dictionary of reactions. However, most of them are cumbersome to separate from the synthetic mixture. Immobilisation of such metal catalysts on polymeric supports, leading to heterogeneous catalysis would thus avoid its contamination of the synthesized products. Unlike homogeneous catalysts, these polymer supported heterogeneous catalysts are recyclable and active after one use and hence economical and environmentally friendly - a feature that has been appreciated by environmental chemists.

Many transformations have been reported using this technique of metal catalyst immobilization.¹⁴⁶⁻¹⁶³

Polymer supported metal catalyzed transformations can surpass the traditional isolation techniques of solvent extraction and column chromatography in a variety of transformations by virtue of their heterogeneous catalytic nature. Thus, polymer supported metal catalysts have opened up a novel field for the synthesis of a plethora of functionally diverse molecules in an efficient, cost and time effective manner.

By the turn of a couple of years, polymer supported catalyst and substrates might as well displace the need for time consuming isolation techniques and pave avenues for greater and more efficient methodologies for organic synthesis. With a number of methodologies already available at our disposal for literally every kind of transformation, such polymer supported techniques would assist in suitably engineering reaction conditions, for the synthesis of *desired compounds*. As Prof. Barton said, "Hundred years of Synthetic Organic Chemistry has taught us as to *How to make*. The question now is *what to make*". The ease in synthesis of active polyaniline and polytoluidine¹⁶⁴ and their impregnation with metal catalysts attracted our early attention. It has been reported from our group that Cobalt complexes efficiently catalyse the epoxidation of electron deficient α,β -unsaturated esters and styryl systems.^{165, 166}

Polyaniline supported Cobalt catalysts are synthesised by mixing equal amounts (by weight) of polyeucoemeraldine base and the cobalt catalyst (cobalt (II) salen and cobalt (II) acetate in the present study) in a 1:1 mixture of acetic acid and acetonitrile at ambient temperature for 36h. Filtering, washing with acetonitrile and drying yield the polymer supported cobalt catalyst. The structure of polyaniline supported cobalt salen has been proposed by us as follows:

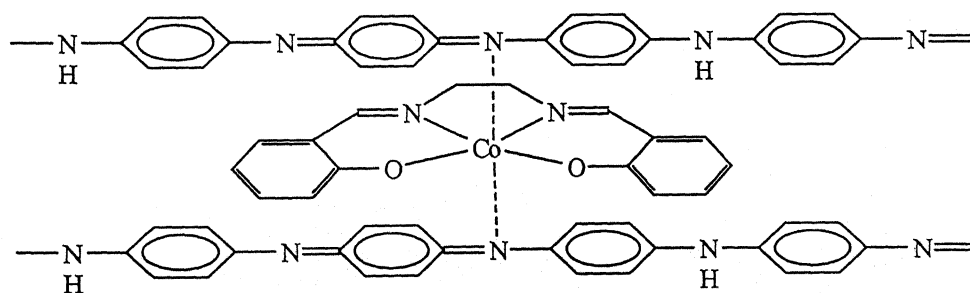


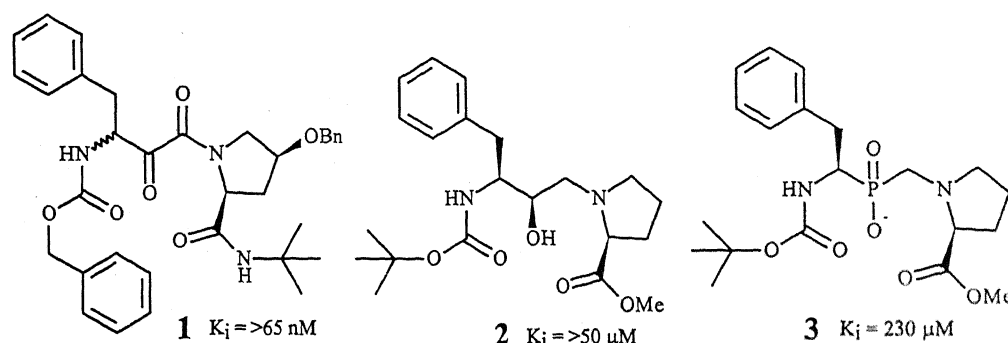
Figure-39

Present Study

α -Hydroxymethylenecarbonyl and α -Keto Amide Isosteres

The transition state mimetic isosteres, especially α -keto and hydroxymethyl carbonyl, have been successfully incorporated as part of small peptides at the scissile bond sites of enzyme bound substrates to arrive at highly potent inhibitors of HIV PR. In our effort to design and synthesize small molecule peptidomimetics, through a cost effective methodology as leads against HIV PR, our focus was fixed on the Phe-Pro scissile peptide bond, uniquely cleaved by the HIV PR.

Inspired by the high potencies of molecules containing the α -keto amide at the scissile site reported by many groups,^{16,167} we chose the α -keto amide moiety as the isostere. This is the most advantageous isostere, owing to the ease of its synthesis due to lack of stereochemical requirements. It is known that the α -keto group binds to the two aspartyl moieties Asp₂₅ and Asp₁₂₅ of the HIV PR non-covalently and thus mimics the transition state of the substrate bound HIV PR complex.



Comparison of α -Keto Amide Core Structure with Other Isosteric Structures

Figure-1

Need for Complimentary Groups Away From Scissile Site

However, Chi-Huey Wong had proposed that the presence of new complimentary groups "away from" the scissile site would increase the binding ability of such ligands¹⁶ (Figure-2). Fairlie *et al* noted that¹⁶⁸ greater binding of their cyclic inhibitors was seen by creating the cycle one atom removed from the hydroxyethylamine nitrogen, rather than incorporating the nitrogen in the cycle (Figure-3). This rendered the nitrogen sterically unencumbered leading to better interaction with Asp 25 residues of the HIV PR. Another advantage in introducing the binding element such as the α -hydroxymethylenecarbonyl, one carbon away instead of at the α -carbon is that stereochemical stringency at the carbon

bearing the binding element can be reduced, for better binding with the enzyme. The advantage of such a simplification can be realized in context of the need for synthesis of large number of such molecules; hence, lesser the complexities in required molecules, the easier its synthesis.

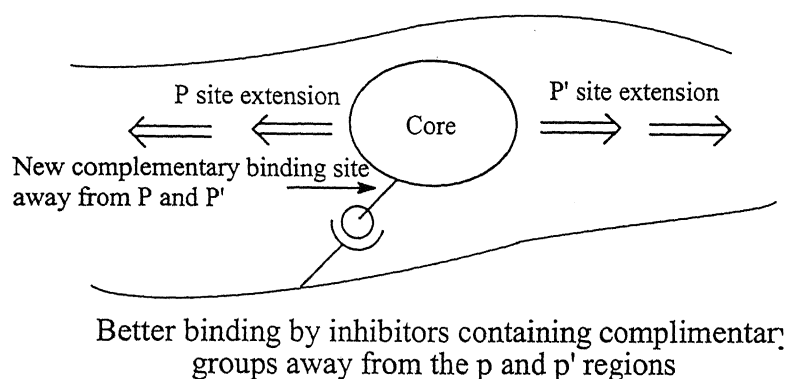
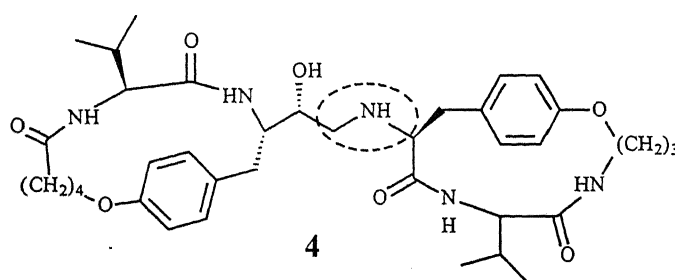


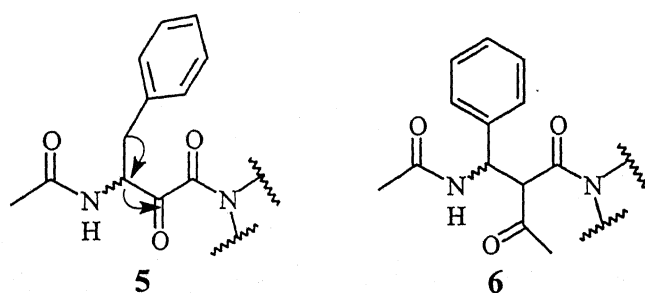
Figure-2



Creating the cyclic peptide one atom removed from the HEA isostere nitrogen, rather than incorporating the nitrogen in the cycle, led to better binding

Figure-3

This led us to believe that homologation of the carbonyl by one methylene would help to bring it closer to the Aspartyl groups hence assisting in greater binding.

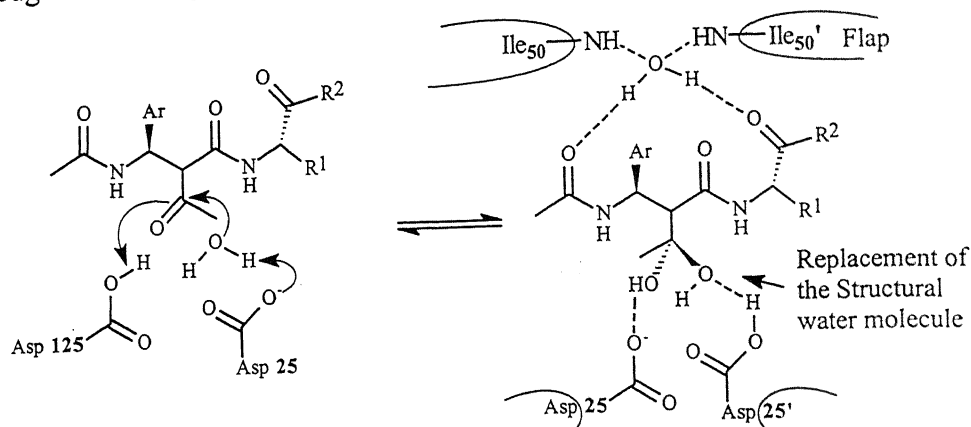


Homologation of the ketone carbonyl in α -ketoamide isostere containing peptidomimetics (1) to get to (2)

Figure-4

Since aldehydes are highly susceptible to chemical reactions en-route to the target sites, ketone was the obvious choice. The simplest of ketones, was chosen. Not many reports

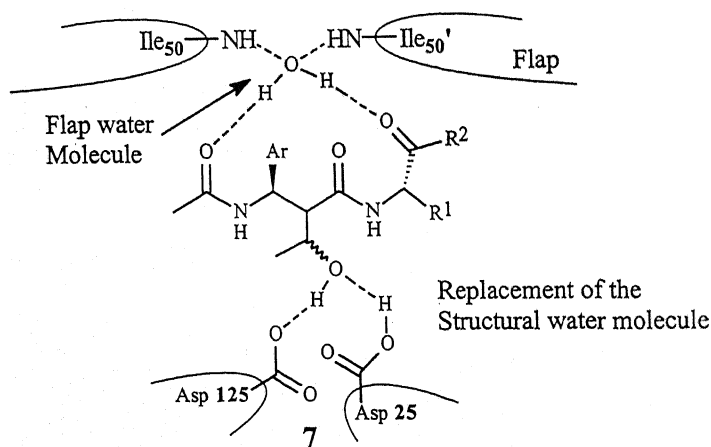
have discussed the replacement or modification of the P₁-Phe residue. Hence it seemed fair enough to homologate the benzyl group to phenyl group.



Schematic representation of proposed general acid-general base catalysed mechanism for the binding of β -acetamido ketone, similar to α -keto amide isostere with HIV PR aspartate groups

Figure-5

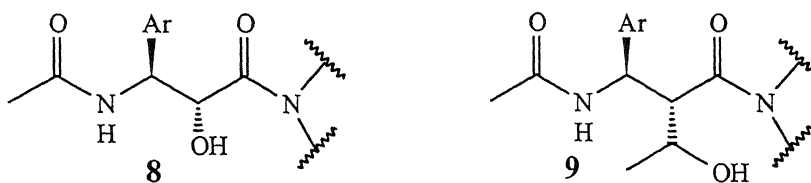
Reduction of the homologated carbonyl would lead to the corresponding hydroxy compound, whose stereochemical requirements for binding with the Asp of HIV-1 would be much less stringent due its location away from the core of the P₁ - P₁' binding site (Figure-6), as discussed earlier.



Schematic representation of the proposed hydrogen bonding interactions between the β -hydroxy β -acetamido isostere and active-site aspartates of HIV PR

Figure-6

The reduced form, we propose would serve to mimic the hydroxymethyl carbonyl isostere (Figure-7).



α -HMC Isostere and Its Surrogate, The Novel
 β -Hydroxy- β -Acetamido Carbonyl Isostere

Figure-7

In their communications, different groups^{9,112,142} have reported the high potency showed by certain structure-based inhibitors, namely 4-hydroxy coumarins and 4-hydroxy-2-pyranones as non-peptidic inhibitors of HIV PR. Lead optimization of this class of

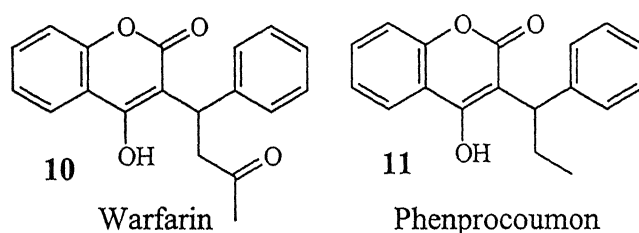
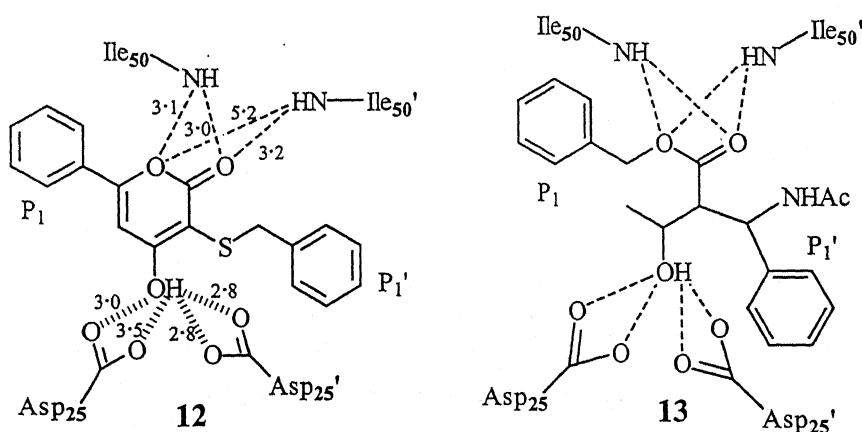


Figure-8

protease inhibitors followed⁹ the report of warfarin and phenprocoumon (the first of this kind of inhibitors).

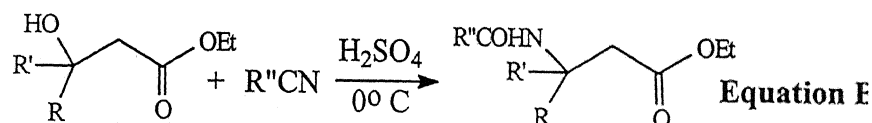
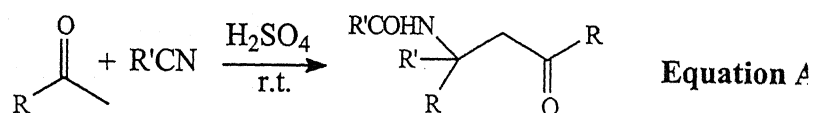
Varaprasad *et al*, later showed¹¹² that in the less rigid δ -lactone 12, the lactone carbonyl strongly bound to the Ile₅₀ and Ile₁₅₀ of the flaps replacing the structural water and the hydroxyl group mimicked the hydroxyethyleneisostere moiety (Figure-9), forming H-bonds with Asp₂₅ and Asp₁₂₅ of the HIV Protease (X-ray crystal structure).



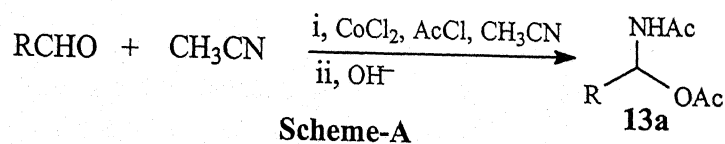
Interatomic distances of compound A binding to HIV PR active-site a
the proposed binding interactions of the β -acetamido ester analogue

Figure-9

The remarkably striking resemblance of these class of inhibitors¹¹² to the homologated α -ketoamide isosteres led us to reason that since the lactone carbonyls bound better to the flap Ile molecule¹¹² in the absence of the rigid fused phenyl ring, the entropic gain in the molecule in going from the cyclic- β -hydroxy- δ -lactone **12**, to the acyclic benzyl ester derivative **13** might be very little. Moreover, the compound **12** has been reported to show only millimolar inhibitory potency against HIV-PR. Hence, the better availability of the carbonyl in the acyclic system **13** for probable hydrogen bonding due to its greater degree of rotational freedom than its lactone counterpart could be expected to counter the entropic gain in **12** by virtue of its cyclic structure and thus lead to better binding of **13** than **12**. Thus, we set out to synthesize the novel class of β -ketoamide isostere containing small molecule peptidomimic β -amino acids **6** as probable leads for the inhibition of HIV PR. A brief review into the literature on existing methodologies for the synthesis of β -amino acids¹⁶⁹⁻¹⁷⁵ revealed that most of the syntheses involve multistep synthetic routes, a feature which dampens their applicability in the present context. However, it was interesting to observe reports¹⁷⁶⁻¹⁷⁷ for the synthesis of β -acylamino ketones and esters by the condensation of ketones/esters with nitriles in the presence of concentrated sulphuric acid (Equations A, B).



Prompted by i) these reports; ii) earlier observations in our group that aldehydes react with acetylchloride in acetonitrile in the presence of catalytic amounts of CoCl_2 to afford α -acetoxy amides **13a** in high yields (Scheme-A); and the fact that metal catalysed

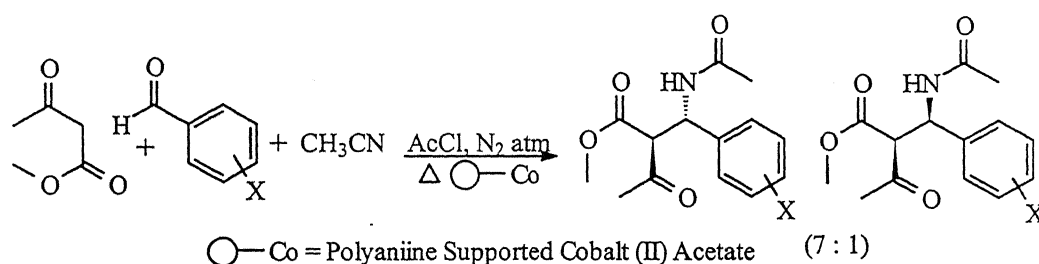


Scheme-A

coupling between enolisable ketones and aldehydes are rare in literature for the formation of corresponding aldol related β -keto amino derivatives; we had demonstrated¹⁷⁸ that cobalt (II) chloride catalyses the coupling between an enolisable ketone or ketoester, an

aldehyde and acetonitrile in the presence of acetylchloride to provide a general route to the synthesis of β -acetamido carbonyl compounds.

In order to broaden the synthetic scope of this reaction, we explored the possibility of developing this reaction via a combinatorial approach leading to a general synthesis of β -amino acid derivatives by applying a polymer supported cobalt catalyzed three component coupling protocol. It has been demonstrated by us¹⁷⁹ that polyaniline supported cobalt (II) acetate or cobalt (II) salen functions as an efficient catalyst in promoting the epoxidation of alkenes and their subsequent opening by aniline or its derivatives. We now demonstrate that polyaniline supported cobalt (II) acetate catalyzes the coupling between methyl acetoacetate, an aldehyde and acetonitrile to provide a general synthetic route to β -amino acid derivatives (Scheme-1), hence leading to a library of small molecule peptidomimetics as probable, potent inhibitor ligands against HIV-PR



Scheme-1

This transformation however is influenced by the presence of molecular oxygen, which causes the formation of α β -unsaturated carbonyl compounds (Knoevenagel product) along with the expected β -acetamido carbonyl compounds. It was observed that alteration of the ratio of amides and enones during the reaction could be obtained at ambient temperature. However, the time scales of such reactions were observed to be very high for the completion of reaction. On the other hand, a moderate to good yield of β -acetamido carbonyl compounds were obtained under nitrogen atmosphere which suppressed the formation of the α , β -unsaturated carbonyl compounds to a large extent as only traces of the later were observed in the crude reaction mixture. The most important feature of this methodology is the simple isolation procedures that follow the synthesis of β -acetamido ketones. Thus, filtration of the catalyst and washing the contents of the concentrated reaction mixture with CCl_4 -hexane solvent system yielded the polar products (most of them are good solids) in good yields. These reactions have been

performed in up to 25 millimolar (mmol) scale and the products were isolated in pure form (~95-100%) without subjecting the reaction mixture to column chromatography.

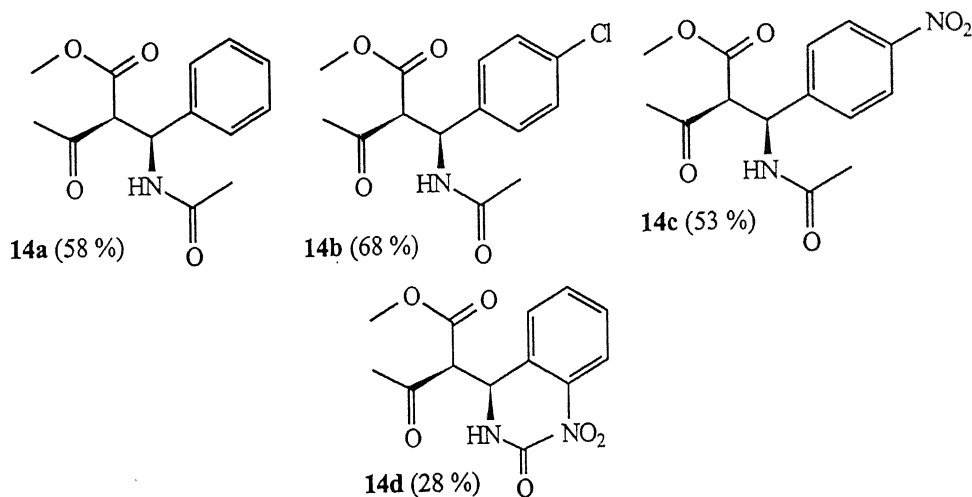


Table-1

The reaction of methyl acetoacetate with benzaldehyde, *para*-chloro-benzaldehyde, *para*-nitrobenzaldehyde and *ortho*-nitrobenzaldehyde and 3-methylbutanal proceeded smoothly in the presence of polyaniline supported cobalt (II) acetate to afford the corresponding β -acetamido esters in good yields (Table-1) (Note: Yields have been reported with respect to the initial amount of ketone/ acetoacetate taken for reaction). In the case of *ortho*-nitrobenzaldehyde, the Knoevenegal product (enone- **14d***) was formed in substantial amounts (20%). Trace amounts (~5%) of the corresponding enone was also formed on reaction with *para*-nitrobenzaldehyde.

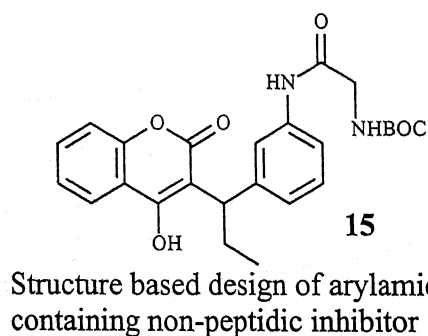


Figure-10

Henry Skulmick *et al* had reported¹⁴² their observations that the presence of an amide linkage (Figure-10) to the aromatic P₁' site, could improve the potency of the coumarin

based inhibitors.¹⁸⁰ They reported the presence of additional hydrogen bonds in the enzyme-bound X-ray crystal structure of **15**, which were absent in the parent compounds (Warfarin, Phenprocoumon).

Extending this observation into our polyaniline supported cobalt (II) acetate catalysed MCC methodology, we found that *ortho*-, *meta*- and *para*-hydroxy benzaldehydes in the form of salicylaldehyde, vanillin and isovanillin, reacted with methyl acetoacetate in the presence of polyaniline supported cobalt (II) acetate to yield the corresponding aryloxy acetylated β -acetamido esters, in good yields. (Table-2). Except in the case of **14g**, which was wrought with the formation of subsequent amounts of the enone (**14g***), all other reactions proceeded smoothly with no traceable amounts of side products. Attempts to modify experimental conditions for the formation of **14g** did not suppress the formation of the side product.

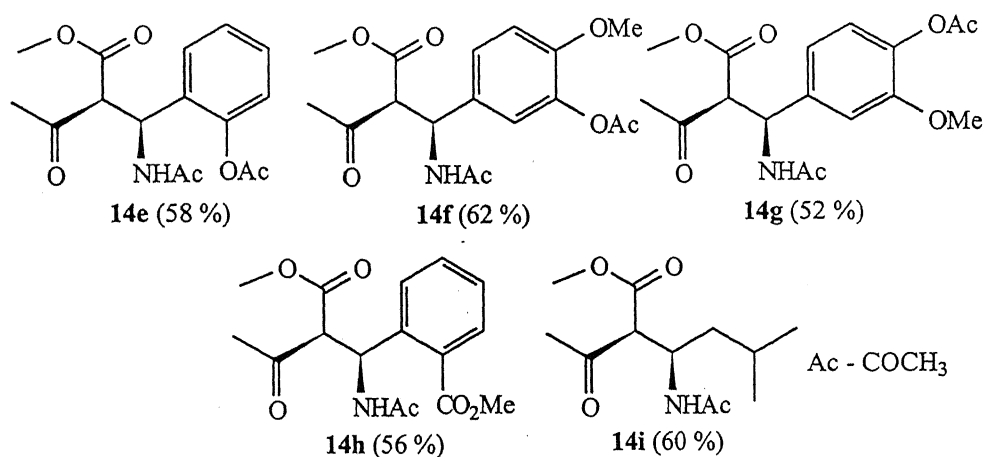
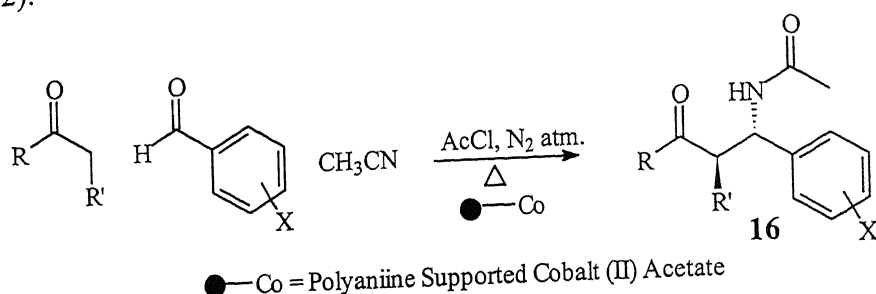


Table-2

Ortho-(methylformyl)benzaldehyde reacted with acetoacetate in these conditions to yield the corresponding β -acetamido ester in good yields. While in general efforts to replace the P₁' aromatic residue with aliphatic side chains was crippled by the non-reactivity of aliphatic aldehydes with acetoacetate under similar conditions, 3-methylbutanal gave the corresponding acetamido esters in the presence of polyaniline supported cobalt (II) acetate (Table-2).

These compounds were obtained as a mixture of *syn* and *anti* diastereomers in which the *anti* diastereomer was found to be the major (\sim >7:1) product, concluded from a comparison of the ¹H NMR coupling constants of the benzyl methines.

α-substituted ketones also react with aromatic aldehydes under these conditions to afford the corresponding more substituted β-acetamido ketones in a diastereoselective manner (Scheme-2).



Scheme-2

Ethylmethyl ketone reacted with benzaldehyde, *para*-chlorobenzaldehyde and Vanillin to yield the β-ketoamides (**16a-c**) in moderate to good yield.

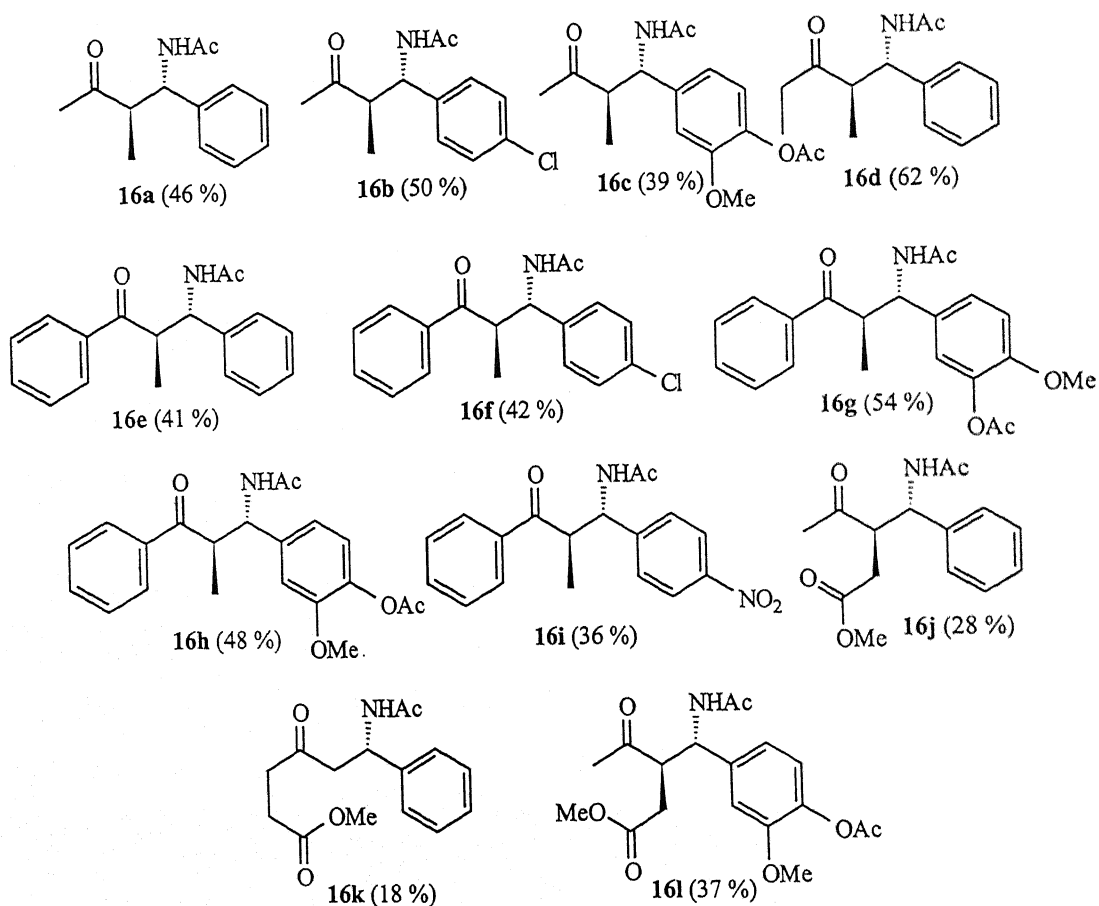


Table-3

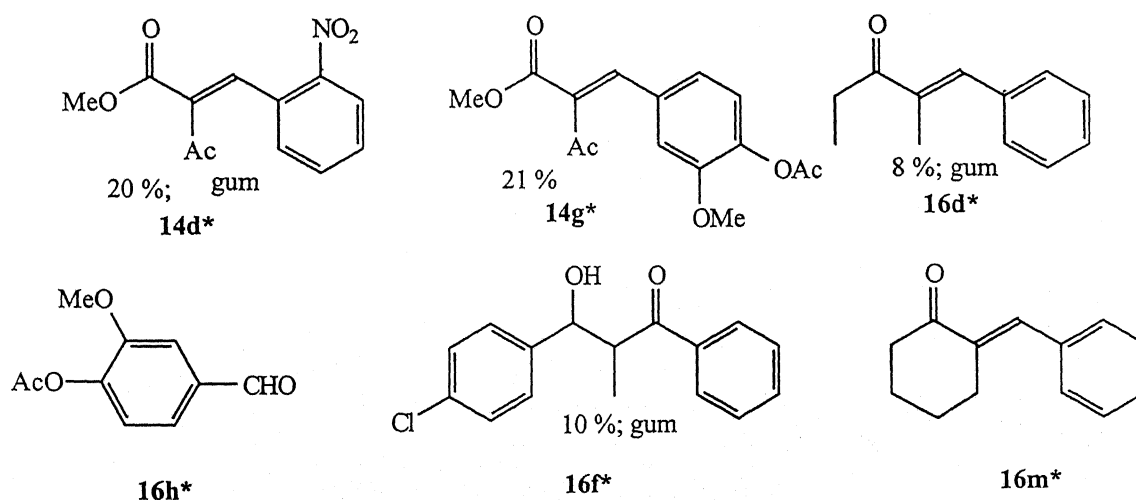
Propiophenone gave good yields of β-acetamide ketones (**16e-i**) in good yields on reaction with benzaldehyde, *para*-chlorobenzaldehyde, isovanillin, vanillin, and *para*-nitrobenzaldehyde respectively. However, examination of the CCl₄-hexane wash revealed

the formation of the aldol **16f*** and acyl vanillin **16h*** as minor side products during the synthesis **16f** and **16h** respectively. While methyl levulinate reacted with vanillin to give **16l** as a single product in reasonable yields, the reaction with benzaldehyde yielded a mixture of regio isomers **16j** and **16k** in very moderate yields. Para-chlorobenzaldehyde and para-nitrobenzaldehyde did not react under identical conditions with methyl levulinate. While diethylketone yielded **16d** in encouraging yields on reaction with benzaldehyde, it's CCl_4 -hexane wash showed formation of the enone **16d*** in small amounts (8%).

However, reactions with benzaldehyde, para-chlorobenzaldehyde and vanillin with the symmetric ketones, diethylketone and cyclohexanone did not yield the corresponding β -ketoamides. While **16m*** was formed on reaction of cyclohexanone with benzaldehyde, the reaction mixtures of other reactions revealed the recovery of unreacted starting material, upon water and base wash.

Aliphatic aldehydes, in general seem to be less suited for the reactions for the formation of the corresponding β -ketoamides as exemplified by the non-reactivity of 2-methylpropanol, n-butanal, crotonaldehyde and cinnamaldehyde with the above mentioned enolisable ketones.

Among aromatic aldehydes, para-methoxy benzaldehyde was the odd-man-out in being adamant to undergo reactions with the ketones, unlike its other counterparts (we have not made attempts to rationalize this observation).



Part A Section I

However, in general, aromatic aldehydes seem to possess the optimum electronic requirements for good reactions by PASCOA catalysed protocol for the formation of β -ketoamides from reactive ketones.

As in the case with acetoacetate, in all the cases, the *anti* diastereomers were formed as the major products, which were isolated by column chromatography, along with minor amounts (~ 10-15%) of the *syn* diastereomer (NMR). The chiral HPLC of **14g**, **14i** and **16h** have been presented in the annexure for exemplification.

Experimental Conditions


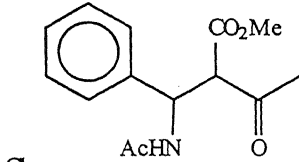
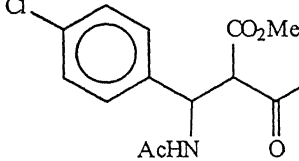
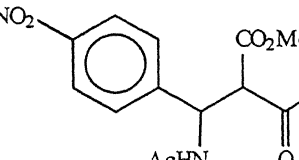
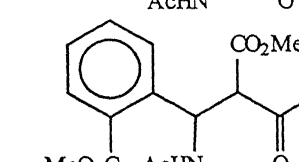
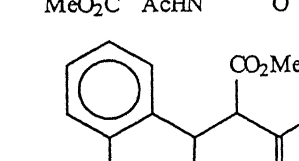
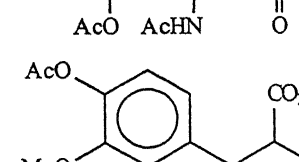
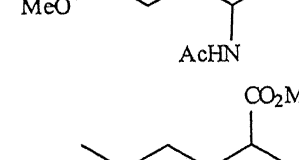
The optimum reaction conditions and general procedure for the synthesis of β -acetaetamidoketones, have been presented in the experimental section. In general, maintenance of strict nitrogenous atmosphere throughout the reaction was an essential factor to suppress the formation of side products. It is best to cool the reaction flask to room temperature before checking for progress of reaction on TLC, to avoid formation of side products. TLCs can be conveniently visualized in the I_2 chambers. Addition of greater amounts of acetylchloride (than mentioned) did not increase the yield of products, unless mentioned otherwise.

Comparison of Diastereoselectivity in Polyaniline-Supported Cobalt (II) Acetate and Cobalt (II) Chloride Catalysed Synthesis of β -Acetamido Carbonyl Compounds


| β -Acetamido ketone | Syn : Anti ^a | |
|---------------------------|---------------------------------|--------------------------------------|
| | \ominus Co catalysed reaction | CoCl ₂ Catalysed reaction |
| | 7.5 : 1 | 3 : 1 |
| | >10 : 1 | 3 : 1 |
| | 7.5 : 1 | 3 : 1 |

^aRatio obtained from HPLC (Annexure) of the crude mixture

**Polyaniline supported Cobalt (II) Acetate and Cobalt (II) Chloride
Catalysed Synthesis of β -Amino Acid Derivatives: A comparative Study**

| β -Amino acid derivative | Yield (%) obtained from | | M.P. ^e |
|-------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------|-------------------|
| |  Co catalysed ^{a,b} reaction | CoCl ₂ catalysed ^{c,d} reaction | |
|  | 62 | 48 | 129-31 °C |
|  | 68 | 41 | 130-32 °C |
|  | 53 | 31 | 149-51 °C |
|  | 56 | 41 | 145-46 °C |
|  | 58 | 41 | .. ^f |
|  | 51 | 43 | 173-75 °C |
|  | 55 | 52 | .. ^f |

^aYield of the product purified by CCl₄-hexane wash; ^bTrans diastereomer was obtained as the predominant product (*cis-trans* = 1:>7); ^cYield of the product obtained by aqueous workup and column chromatography; ^dThe product is obtained as a 3:1 mixture of diastereomer; ^eMelting point of the trans diastereomer; ^fThis compound was obtained as a gum.

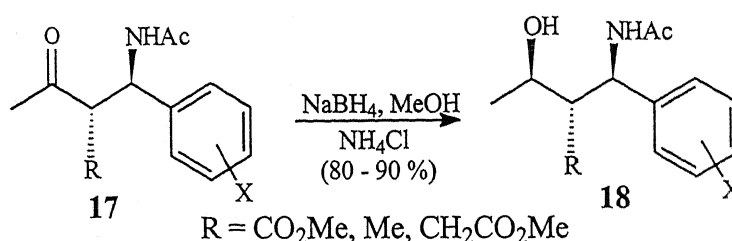
 Co = polyaniline-supported cobalt (II) acetate

However, addition of excess of acetylchloride could lead to poorer yield of the required product. Refluxing the reaction contents for more than the mentioned times did not increase or decrease the yield of required products. Addition of stoichiometric amounts of

aldehydes was optimum for reaction. In the PASCOA catalyzed reactions between ketones and aldehydes, addition of fresh catalyst was not necessary during the reaction. For the cobalt (II) chloride catalyzed reactions, better yields were realized on addition of the catalyst in two lots, one in the beginning and the other after ~8h of the reaction. In the case of hydroxyaldehydes, performing the reaction in slight excess (5 mL/mmol of ketone) of acetonitrile resulted in better yields of required products. Similarly, five equivalents of acetylchloride was required for the reactions involving hydroxyaldehydes. Better isolations were observed by rapid washing under suction through a G-3/G-4 sintered funnel rather on filter paper. As one repeats the wash (see experimental procedure for details) with CCl_4 -hexane, reduction in the amount of CCl_4 taken and the use of warm or hot hexane resulted in better isolations.

Novel Surrogates for the HMC Isostere

The *anti* β -ketoamides thus synthesised, underwent stereoselective reduction to the corresponding α -hydroxyethyl β -amino acids, the β -arylhomoisothreonine derivatives, smoothly. As discussed earlier, we propose these β -arylhomoisothreonine derivatives to be novel surrogates for the highly potent hydroxymethylcarbonyl (HMC) containing dipeptide isosteres, containing the hydroxyethylene moiety "away" from the core structure.



Scheme-3

Thus sodium borohydride reduction of the β -acetamido ketones (**14a-c,h,i,16a,j**) in methanol afforded the corresponding β -arylhomoisothreonine derivatives (**18a-g**) mainly as 1,3-*syn*-diastereomers along with minor amounts (~10%) of the corresponding 1,3-*anti*-diastereomers, in all the cases (Scheme-3). Reduction of **16j** yielded the cyclic lactone **18g** as the major product, along with minor side products in insignificant amounts.

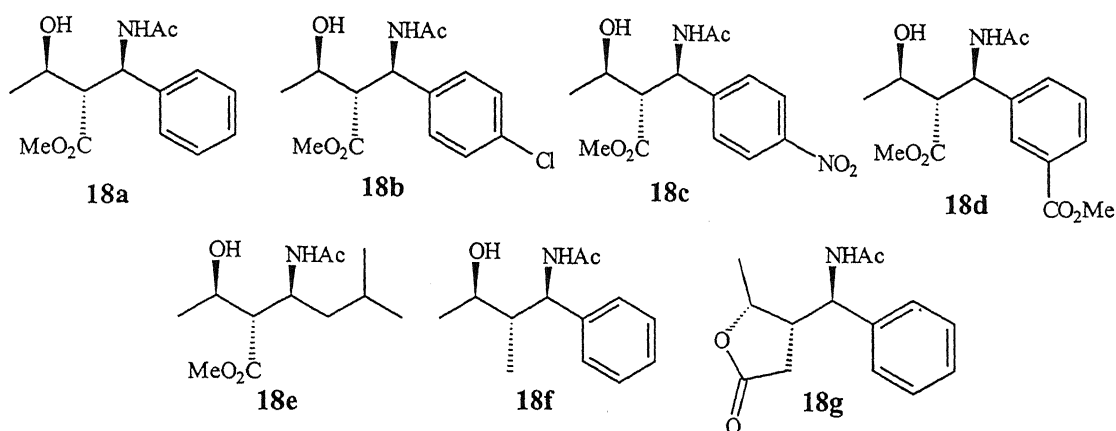


Table-4

This diastereoselectivity may be arising due to the chelated cyclic six-member intermediate, which would result in a 1,3-stereocontrol. The 1,3-*syn* stereochemistry assigned based on the ^1H NMR, where the large coupling constant between the meth protons at C-2 and C-3 is indicative of *anti* stereochemistry between hydroxy/acetamido and methoxycarbonyl groups.

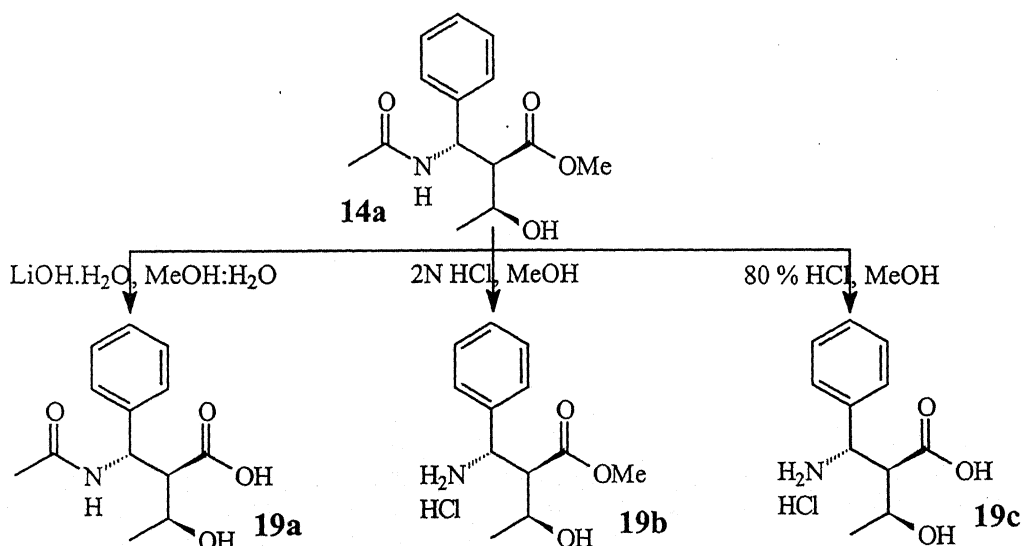
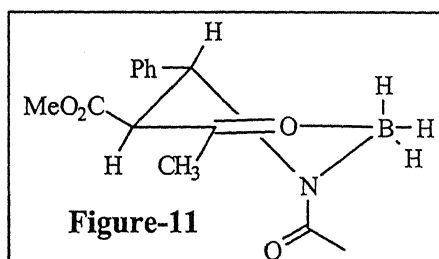


Table-5

Selective deprotection of the N- and C- terminals of these small molecule dipeptide isosteres is possible under acid and base hydrolysing conditions respectively.

Thus, base hydrolysis of **14a** with potassium hydroxide in methanol-water mixture, gave the β -acetamido carboxylic acid; and acid hydrolysis with 2N HCl in methanol yielded the free β -amino ester in good yields. Complete hydrolysis to the corresponding amino acid occurred by refluxing in 80 % HCl in methanol.

Several N-terminal protecting groups were incorporated as lead optimised peptidomimetics, based on similarly reported potent dipeptide isosteres. Thus, the reaction of the β -amino ester with cinnamoyl chloride, benzoyl chloride and methylchloroformate yielded the corresponding N-protected amido esters in good yields (Table-6).

Lead Optimized Small Molecule Isosteres

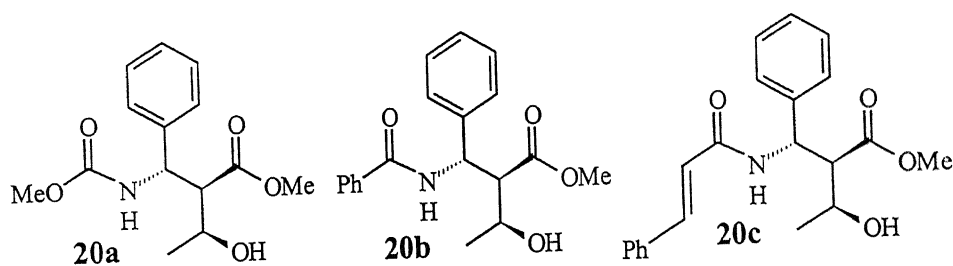
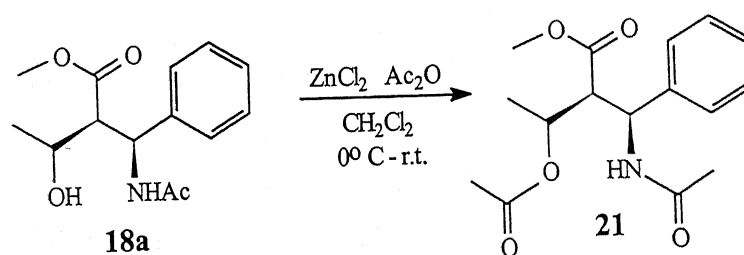
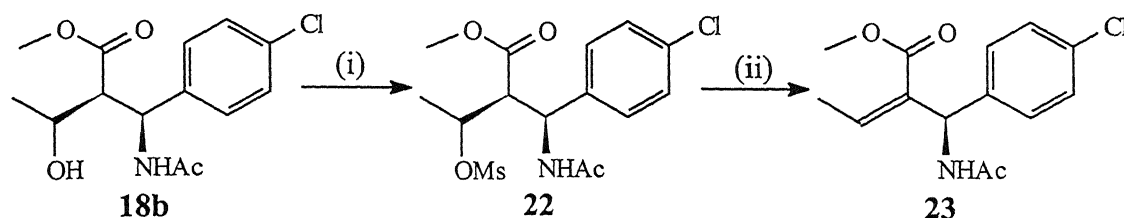


Table-6

The secondary alcohol **18a** reacted with acetic anhydride in the presence of zinc chloride (the reaction did not occur in the presence of NEt_3 , pyridine or DMAP) to give the acetylated product **21** in good yields.

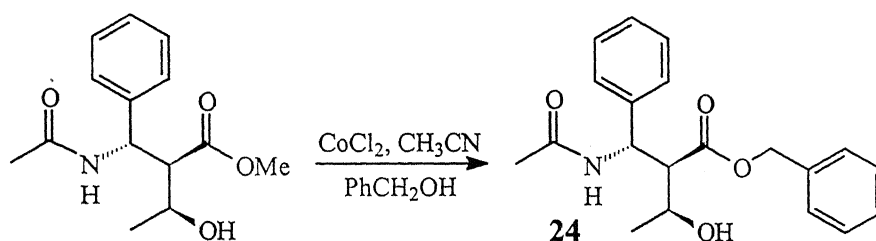


The β -acetamido ketones could be used as synthons for the synthesis of α -dehydro β -aminoacid derivatives. The β -phenylhomoisothreonine derivative **18b** was converted to its corresponding α -dehydro- β -aminoester counterpart **23**, by mesylation of the alcohol to **22**, followed by refluxing in the presence of triethylamine and NaI in acetonitrile.

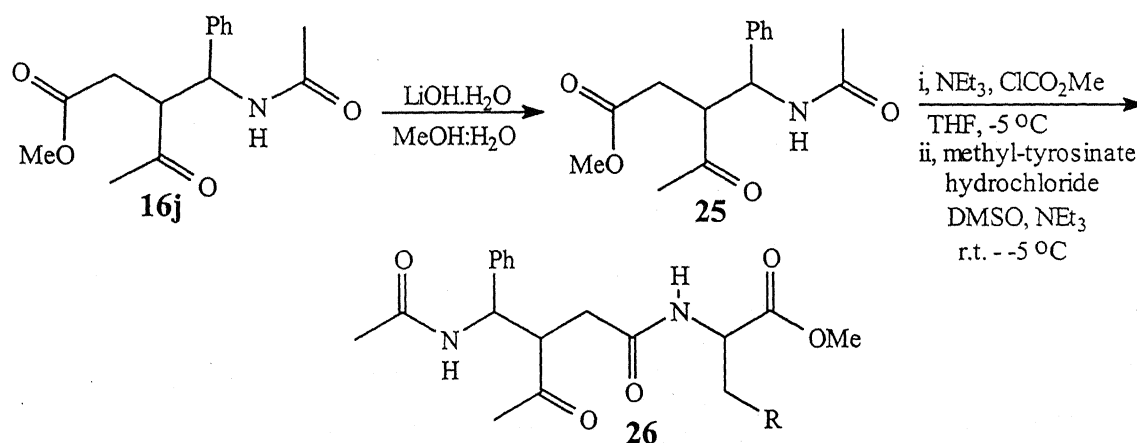


Legend: i, methanesulphonylchloride, pyridine, CH_2Cl_2 ; ii, NEt_3 , NaI , CH_3CN , reflux

The β -acetamido ester **14a** underwent transesterification with benzyl alcohol, in the presence of CoCl_2 in acetonitrile to give the corresponding benzyl ester **21** in good yields. This is an acyclic structural analogue of the highly potent inhibitor **12** reported¹¹² by Vara Prasad *et al.*



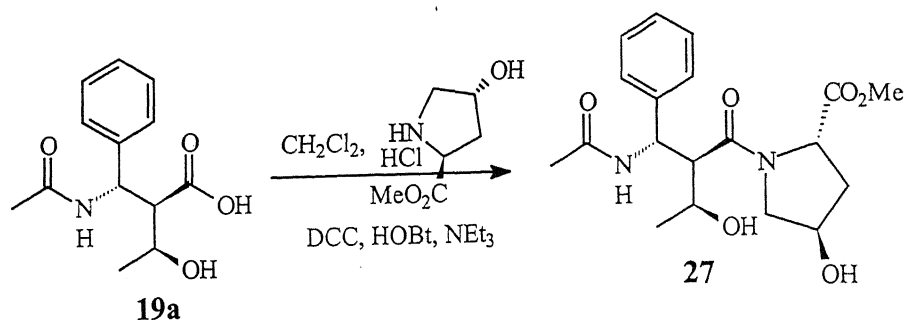
C-terminal extensions could also be done on β -aryl homoisothreonine derivatives. Hydrolysis of **16j** was accomplished with $\text{LiOH} \cdot \text{H}_2\text{O}$, in methanol, to give the corresponding carboxylic acid **25**, which was coupled with tyrosinate hydrochloride using the mixed anhydride coupling procedure with methylchloroformate as the carboxyl activating reagent, to give **26**.



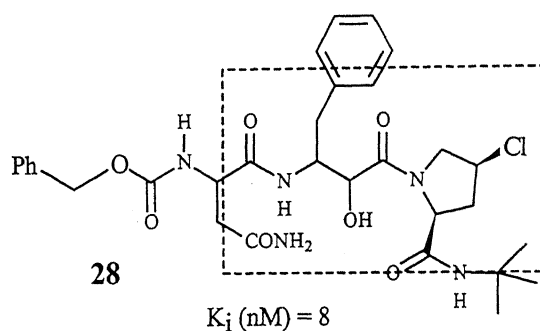
Compound **25** is a Phe-Tyr dipeptide peptidomimic with novel β -acetyl binding element away from the peptide core.

The Phe-Pro peptidomimic **27**, containing the hydroxy ethylene isostere away from the core structure, a partial structural mimic of the compound **28** (with nanomolar inhibitory

potency against HIV PR¹⁰⁵ and hence another highly prospective candidate as drug lead against HIV PR was synthesized.



Reaction of **19a** with methyl-L-(4-*trans*-hydroxy)proline hydrochloride in CH_2Cl_2 in the presence of dicyclohexylcarbodiimide (DCC), 1-hydroxy benzotriazole (HOBT) and triethylamine (TEA) gave the α -hydroxyethylene containing Phe-Pro dipeptide isostere **27** in good yields.



Structure of a HMC isostere containing Phe-Pr Peptidomimic showing nanomolar inhibitory activity against HIV PR

Figure-12

Thus, these β -aryl-homoisothreonines can be incorporated as part of peptide systems as novel isosteres, containing the crucial hydroxy group away from the $\text{P}_n\text{-P}_{n'}$ core structure. In conclusion, we have developed a versatile and simple protocol for the synthesis of library of small molecule peptidomimetics, containing novel isosteres, the α -hydroxyethylene moiety and α -acetyl ketone moiety, away from the peptidomimic core structure - catalysed by polyaniline supported cobalt catalyst. This highly efficient MCC procedure is moderate to good in yields; allowing reactions to be done in gram quantities and employs a non-aqueous purification procedure that circumvents the need for column chromatography. Each of these small molecules is by itself a potential drug lead and the presence of protected N- and C-terminal handles allows the introduction of amino acids

of choice at both ends. Thus, these isosteres can be incorporated as part of peptide systems.

The β -aryl homoisothreonine derivatives, we propose, could be excellent surrogates for the highly potent HMC isostere. To our knowledge this is the first report of its kind, for the design and synthesis of small molecule peptidomimetics containing the binding element to the HIV PR active site "away" from the Pn-Pn' sites, as proposed by Wong et al.¹⁶ We have also synthesized dipeptide isosteres **21**, **23**, **24**, **26** and **27** containing β -phenyl homoisothreonine and α -acetyl amido isostere. Peptidomimics **24** and **27** are structural analogues of known HIV PR inhibitors, **12** and **28** respectively.

The advantages shared by polyaniline supported cobalt catalysed transformation thus far realized by us are, higher turn-over numbers for the catalyst than its non-polymer supported counterpart; recyclisability (leading towards "greener" transition metal chemistry); and easy non-aqueous isolation procedures for the products (an essential factor for the synthesis of substrates for proteases, considering that water-soluble small molecules are better suited for bioavailability).

Experimental Section

Materials and Methods

Acetonitrile, ethyl acetate, hexane, THF and all other solvents were purified by standard procedures. CoCl_2 was purchased from LOBA Indian Limited and dried at 140°C for 4 h before use. All the amino acids were bought from SpectroChem India Limited and used as such. Cinnamoyl chloride, aldehydes, methylchloroformate, $\text{Co}(\text{OAc})_2$, methylacetoacetate, levulinic acid, ketones, were all procured commercially and were purified before use. The aldehydes were distilled/recrystallized before use. Polyaniline supported $\text{Co}(\text{Salen})$ was prepared according to procedure developed in our lab¹⁸². Column chromatography was performed on ACME silica-gel eulant. TLC was performed on ACME silica-gel-G coated glass plates and were visualised under UV lamp (254 nm) or in Iodine chamber. ^1H NMR spectra were recorded using Jeol PMX-60 system, Bruker WP-80, Jeol 300 FTNMR or JNM-LA400 FTNMR machines in $\text{CCl}_4/\text{CDCl}_3$. Chemical shift are given relative to TMS in ppm (δ). Multiplicity is indicated using the following abbreviations: s(singlet), bs(broad singlet), d(doublet), dd(doublet of a doublet), ddd(doublet of a doublet of a doublet), dt(doublet of a triplet) td(triplet of a doublet), q(quartet) and m(multiplet). The FAB mass spectra were recorded on JEOL SX 102/DA 6000 mass spectrometer data system using Argon (6 Kv, 10 mA) as the FAB gas. All the known compounds were characterized by comparing with the literature data. IR spectra were recorded on Perkin Elmer 683 spectrophotometer, using either a neat sample or a solution in $\text{CCl}_4/\text{CH}_2\text{Cl}_2$ and solids were examined as KBr pellets and the values are reported in ν_{max} (cm^{-1}). HPLC analyses were done with Rainin System fitted with Dynamax[®] SD-200 pump and detected with Groton PDA solonet Diode Array Detector.

Preparation of Polyaniline

Freshly distilled aniline 10 mL (109.5 mmol) was dissolved in 125 mL of 1.5 M HCl, and a solution of ammoniumpersulfate (54.8 mmol) in 1.5 M HCl (125 mL) was added to it at 0°C . Since aniline polymerization is strongly exothermic, the oxidant must be added slowly over a period of 1h. After the addition of the oxidant, the reaction was stirred further for 4 h. The polyaniline hydrochloride precipitate was separated by filtration and washed consecutively with water (3X30 mL), methanol (2X25 mL), and diethyl ether (2X15 mL) to remove the oligomers and any of the reaction side products. The polymer was then vacuum-dried until constant mass. Deprotonation of polyaniline hydrochloride was achieved with aqueous ammonia (3 wt%). Deprotonated polymer was again washed with water, methanol, and diethyl ether and dried until constant mass (~3 gm). Polyaniline is quite stable to air and can be stored indefinitely in closed glass vials.

Preparation of Polyaniline supported cobalt (II) acetate (PASCOA)

Cobaltous acetate (200 mg) and polyaniline (200 mg) were added to a solution of acetic acid (25 mL) in acetonitrile (25 mL) and stirred at ambient temperature for 36 h. The resultant catalyst was filtered off and washed first with acetic acid (3X10 mL) and then thoroughly with acetonitrile until the filtrate was colorless. The resulting residue was dried in an air oven at 100°C for 2 h to afford the black (or blackish brown) colored

catalyst. Polyaniline supported cobalt (II) acetate is stable to atmosphere and can be stored indefinitely in closed vials.

General Procedure for the Synthesis of β -keto amides in the Presence of PASCOA:

Procedure A

Polyaniline supported cobalt (II) acetate (~10 mg) was added to a solution of methyl acetoacetate/ketone (5[#] mmol), aldehyde (5 mmol) and acetyl chloride (15 mmol),* in acetonitrile (15 mL) at ambient temperature. Then the reaction mixture was refluxed under nitrogen atmosphere at 85 °C for 10-16 h and at the completion of reaction (no further significant increase in concentration of product spot on TLC), the catalyst was removed by filtration through a sintered funnel under suction. The filtrate was evaporated to afford a residue, which was dissolved in minimum amount of CCl₄ and addition of hexane drop wise to it resulted in precipitation of the corresponding β -keto amide as a solid (in general most of these β -keto amides are good solids). The solid was filtered off on a sintered funnel under suction and the mother liquor was concentrated and subjected to similar washing with CCl₄-hexane mixture, until no noticeable amounts of product (TLC) was left in it. The combined, crude product was washed once again with CCl₄ and hexane and dried under vacuo to yield the corresponding β -keto amides in high purity (95 - 100 % - HPLC), in moderate to good yields (50 - 70 %). In cases where the product was a gum, the resulting residue after filtration of catalyst and concentration of solvent from the filtrate, was taken in ethyl acetate (25 mL) and the organic layer was washed successively with water (2X10 mL), saturated aqueous solution of NaHCO₃ (3X10 mL),** water (2X10 mL) and brine (1X10 mL). Drying over anhydrous Na₂SO₄ and evaporation of solvent gave the crude product, which was subjected to column chromatography (EtOAc-Hexane) to afford the corresponding β -keto amide in good yields.

Note: Reaction yields have been calculated with respect to the initial amount of ketone taken for reaction.

* For aldehydes with hydroxyl groups 5 equivalents of acetyl chloride was added; good results were observed on using freshly distilled acetylchloride every time.

** Direct washing with bicarbonate solution, without preliminary washing with water - of the reaction mixture - is to be avoided as it generates tremendous pressure in the separating funnel.

* Reactions were equally effective and reproducible up to a scale of 15-20 millimoles. Unless mentioned otherwise, the best scale reactions have been reported.

General Procedure for the Synthesis of β -keto amides in the Presence of CoCl₂:

Procedure B

To a solution of methyl acetoacetate/ketone (1[#] mmol), aldehyde (1 mmol) and acetyl chloride (3 mmol)* in acetonitrile (~5 mL) at ambient temperature was added anhydrous CoCl₂ (5 mol %) at ambient temperature. Then the reaction mixture was refluxed under nitrogen atmosphere at 85 °C for 14 to 20 h and at the completion of reaction, the solvent was evaporated under vacuo, and the residue was taken in EtOAc (10 mL) and the organic layer was washed successively with water (1X10 mL), saturated aqueous solution of NaHCO₃ (3X5 mL),** water (2X5 mL) and brine (1X5 mL). Drying over anhydrous Na₂SO₄ and evaporation of solvent gave the crude product, which was purified by

column chromatography (EtOAc-Hexane) to afford the corresponding β -keto amide in good yields.

* For aldehydes with hydroxyl groups, 5 equivalents of acetyl chloride was added.

** Direct washing with bicarbonate solution, without preliminary washing with water -of the reaction mixture - is to be avoided as it generates tremendous pressure in the separating funnel.

Reactions were equally effective and reproducible up to a scale of 15 millimoles.

▯ Cobaltous (II) chloride was dried at 150 °C in hot air oven for 1.5 h just before addition to the reaction vessel.

Synthesis of Methyl- α -acetyl- β -acetamido- β -phenylpropionate (**14a**)

Procedure A

Polyaniline supported cobalt (II) acetate (~10 mg) was added to a solution of methyl acetoacetate (580 mg, 5 mmol), benzaldehyde (0.5 mL, 5 mmol) (freshly distilled) and acetyl chloride (1.1 mL, 15 mmol), in acetonitrile (15 mL) at ambient temperature. Then the reaction mixture was refluxed under nitrogen atmosphere at 85 °C for 10 h and at the completion of reaction, the catalyst was removed by filtration through a sintered funnel under suction. The filtrate was evaporated to afford a residue. The residue was dissolved in minimum amount of CCl₄ and addition of hexane drop wise to it resulted in precipitation of the crude β -keto amide (**14a**) as a solid. The solid was filtered off on a sintered funnel under suction and the mother liquor was repetitively concentrated and subjected to similar washing with CCl₄-hexane mixture, until no noticeable amounts of product was left in it (TLC: EtOAc:Hexane - 2:3; R_f = 0.5). The combined, crude, product was washed once again with CCl₄ and hexane and dried under vacuo to yield **14a** in high purity in good yields (815 mg, 62 %) as a solid (M.P. = 129-31 C). About 10 % of the unreacted acetoacetate was recovered in the wash. There was no noticeable formation of the Knoevenagel product in the reaction. The reaction yield was reproducible up to a scale of 25 mmols.

Procedure B

To a solution of methyl acetoacetate (116mg, 1 mmol), benzaldehyde (0.1 mL, 1 mmol) (freshly distilled) and acetyl chloride (0.22 mL, 3 mmol) in acetonitrile (5 mL) at ambient temperature was added anhydrous CoCl₂ (6 mg, 5 mol %). Then the reaction mixture was refluxed under nitrogen atmosphere at 85 °C for 14 h and at the completion of reaction, the solvent was evaporated under vacuo and the residue was taken in EtOAc (15 mL) and the organic layer was washed successively with water (1X10 mL), saturated aqueous solution of NaHCO₃ (3X5 mL), water (2X5 mL) and brine (1X5 mL). Drying over anhydrous Na₂SO₄ and evaporation of solvent gave the crude product, which was purified by column chromatography (EtOAc-Hexane - 7:13) or CCl₄-hexane wash to afford the β -keto amide **14a** in good (126 mg, 48%) yield as a solid. About 25 % of the starting acetoacetate was recovered, unreacted. Addition of freshly dried catalyst (5 mol %) after 7 h of initial stirring of the reaction mixture, increased the yield by ~5 %. The reaction was reproducible up to a scale of 25 mmols.

¹H NMR (CDCl₃) δ 7.65 (d, J = 10.0 Hz, 1H), 7.34 (s, 5H), 5.84 (dd, J = 12.5, 3.0 Hz, 1H), 4.10 (d, J = 6.0 Hz, 1H), 3.72 (s, 3H), 2.37 (s, 3H), 2.04 (s, 3H); IR (KBr) ν_{max} 3300, 3080, 1720, 1680, 1650, 1095, cm⁻¹; MS *m/z* 263 (M⁺), 147, 130, 105.

Synthesis of Methyl- α -acetyl- β -acetamido- β -*p*-chlorophenylpropionate (14b)**Procedure A**

Polyaniline supported cobalt (II) acetate (~10 mg) was added to a solution of methyl acetoacetate (580 mg, 5 mmol), *p*-chlorobenzaldehyde (703 mg, 5 mmol) and acetyl chloride (1.1 mL, 15 mmol), in acetonitrile (15 mL) at ambient temperature. Then the reaction mixture was refluxed under nitrogen atmosphere at 85 °C for 10 h and at the completion of reaction, the catalyst was removed by filtration through a sintered funnel under suction. The filtrate was evaporated to afford a residue, which was dissolved in minimum amount of CCl₄ and addition of hexane drop wise to it resulted in precipitation of the crude β -keto amide (**14b**) as a solid. The solid was filtered off on a sintered funnel under suction and the mother liquor was repetitively concentrated and subjected to similar washing with CCl₄-hexane mixture, until no noticeable amounts of product was left in it (TLC: EtOAc:Hexane - 2:3; R_f = 0.45). The combined, crude product was washed once again with CCl₄ and hexane and dried under vacuo to yield **14b** in high purity in good yields (1 gm, 68%) as a solid (M.P. = 130-132 °C). About 15 % of the unreacted acetoacetate was recovered in the wash. There was no noticeable formation of the Knoevenagel product in the reaction. The reaction yield was reproducible up to a scale of 25 mmols.

Procedure B

To a solution of methyl acetoacetate (116 mg, 1 mmol), *p*-chlorobenzaldehyde (140 mg, 1 mmol) and acetyl chloride (0.21 mL, 3 mmol) in acetonitrile (5 mL) at ambient temperature was added anhydrous CoCl₂ (6 mg). Then the reaction mixture was refluxed under nitrogen atmosphere at 85 °C for 13 h and at the completion of reaction, the solvent was evaporated under vacuo, and the residue was taken in EtOAc (10 mL) and the organic layer was washed successively with water (1X10 mL), saturated aqueous solution of NaHCO₃ (3X5 mL), water (2X5 mL) and brine (1X5 mL). Drying over anhydrous Na₂SO₄ and evaporation of solvent gave the crude product, which was purified by column chromatography (EtOAc-Hexane - 2:3) or CCl₄-hexane wash to afford the β -keto amide **14b** in good (122 mg, 41 %) yield as a solid (M.P.). About 25 % of unreacted acetoacetate was recovered. The reaction was reproducible up to a scale of 20 mmols.

¹H NMR (CDCl₃) δ 7.62 (d, J = 10.0 Hz, 1H), 7.7-7.1 (m, 5H), 5.70 (dd, J = 10.0 and 5 Hz, 1H), 4.01 (d, J = 6.25 Hz, 1H), 3.58 (s, 3H), 2.30 (s, 3H), 2.02 (s, 3H); IR (KBr) ν_{\max} 3300, 1735, 1715, 1650, 1435, 1370, 1095, 820 cm⁻¹; MS m/z 298 (M⁺), 182, 165, 140.

Synthesis of Methyl- α -acetyl- β -acetamido- β -*p*-nitrophenylpropionate (14c)**Procedure A**

Polyaniline supported cobalt (II) acetate (~5 mg) was added to a solution of methyl acetoacetate (580 mg, 5 mmol), *p*-nitrobenzaldehyde (756 mg, 5 mmol) and acetyl chloride (1.1 mL, 15 mmol), in acetonitrile (15 mL) at ambient temperature. Then the reaction mixture was refluxed under nitrogen atmosphere at 85 °C for 16 h and at the completion of reaction, the catalyst was removed by filtration through a sintered funnel under suction. The filtrate was evaporated to afford a residue, which was dissolved in minimum amount of CCl₄ and addition of hexane drop wise to it resulted in precipitation of the crude β -keto amide (**14c**) as a solid. The solid was filtered off on a sintered funnel under suction and the mother liquor was repetitively concentrated and subjected to similar washing with CCl₄-hexane mixture, until no noticeable amounts of product was left in it (TLC: EtOAc:Hexane - 1:1; R_f = 0.40). The combined, crude product was

washed once again with CCl_4 and hexane and dried under vacuo to yield **14c** along with the Knoevenegal product. The mixture of products was subjected to column chromatography to yield the β -keto amide **14c** in moderate yields (816 mg, 53 %) as a solid (M.P. = 149 - 151 °C). About 20 % of the unreacted acetoacetate was recovered in the wash. Addition of additional amounts of acetylchloride or catalyst or increased reaction time did not improve the yields or conversion significantly. The Knoevenegal product was formed in small amounts (~5 %) in the reaction as a side product. The reaction yield was reproducible up to a scale of 10 mmols.

Procedure B

To a solution of methyl acetoacetate (116 mg, 1 mmol), *p*-nitrobenzaldehyde (151 mg, 1 mmol) and acetyl chloride (0.22 mL, 3 mmol) in acetonitrile (5 mL) at ambient temperature was added anhydrous CoCl_2 (~6 mg). Then the reaction mixture was refluxed under nitrogen atmosphere at 85 °C for 15 h and at the completion of reaction, the solvent was evaporated under vacuo, and the residue was taken in EtOAc (10 mL) and the organic layer was washed successively with water (1X10 mL), saturated aqueous solution of NaHCO_3 (3X5 mL), water (2X5 mL) and brine (1X5 mL). Drying over anhydrous Na_2SO_4 and evaporation of solvent gave the crude product, which was purified by column chromatography (EtOAc-Hexane - 2:3) or CCl_4 -hexane wash to afford the β -keto amide **14c** in very moderate (95 mg, 31 %) yield as a solid. About 40 % of unreacted acetoacetate was recovered. The reaction was reproducible up to a scale of 15 mmols. The Knoevenegal product was formed in small amounts (~8 %).

^1H NMR (CDCl_3) δ 8.19 (d, J = 9 Hz, 2H), 7.85 (d, J = 7.5 Hz, 1H), 7.52 (d, J = 9 Hz, 2H), 5.90 (dd, J = 12.0, 3 Hz, 1H), 4.14 (d, J = 6.0 Hz, 1H), 3.76 (s, 3H), 2.19 (s, 3H), 2.09 (s, 3H); IR (KBr) ν_{max} 3290, 3050, 1740, 1715, 1675, 1535, 1340, 1100, 850 cm^{-1} ; MS m/z 308 (M^+), 192, 176, 150.

Synthesis of Methyl- α -acetyl- β -(*o*-nitrophenyl)- β -acetamido ester (**14d**)

Polyaniline supported cobalt (II) acetate (~10 mg) was added to a solution of methyl acetoacetate (580 mg, 5 mmol), *o*-nitrobenzaldehyde (756 mg, 5 mmol) and acetyl chloride (1.1 mL, 15 mmol), in acetonitrile (15 mL) at ambient temperature. Then the reaction mixture was refluxed under nitrogen atmosphere at 85 °C for 20 h and at the completion of reaction, the catalyst was removed by filtration through a sintered funnel under suction. The filtrate was evaporated to afford a residue, which was dissolved in minimum amount of CCl_4 and addition of hexane drop wise to it resulted in precipitation of the crude β -keto amide (**14d**) as a solid. The solid was filtered off on a sintered funnel under suction and the mother liquor was repetitively concentrated and subjected to similar washing with CCl_4 -hexane mixture, until no noticeable amounts of product was left in it (TLC: EtOAc:Hexane - 3:2; R_f = 0.50). The combined, crude product was washed once again with CCl_4 and hexane and dried under vacuo to yield **14d** along with the Knoevenegal product. The mixture of products was subjected to column chromatography to yield the β -keto amide **14d** in poor yields (430 mg, 28 %) as a solid (M.P. = 136 - 137 °C). About 50 % of the unreacted acetoacetate was recovered in the wash. Addition of additional amounts of acetylchloride or catalyst or increased reaction time did not improve the yields or conversion significantly. The reaction yield was reproducible up to a scale of 10 mmols. The Knoevenegal product was isolated as a gum in 20 % yield.

14d ^1H NMR (CDCl_3) δ 7.92 (d, J = 8.8 Hz, 1H), 7.75 – 7.32 (m, 4H), 6.34 (dd, J = 9 Hz & 2.5 Hz, 1H), 4.35 (d, J = 4 Hz, 1H), 3.66 (s, 3H), 2.42 (s, 3H), 2.00 (s, 3H)
IR ν_{max} 3270, 3080, 1710, 1690, 1650, 1600

14d* ^1H NMR (CDCl_3) δ 8.2 (d, J = 8 Hz, 2H), 7.78 - 7.58 (m, 2H), 7.31 (s, 1H), 3.90 (s, 3H), 2.36 (s, 3H)

Synthesis of Methyl- α -acetyl- β -(*o*-oxyacetylphenyl)- β -acetamido ester (**14e**)

Procedure A

Polyaniline supported cobalt (II) acetate (~10 mg) was added to a solution of methyl acetoacetate (580 mg, 5 mmol), salicylaldehyde (611 mg, 5 mmol) and acetyl chloride (1.85 mL, 25 mmol), in acetonitrile (25 mL) at ambient temperature. Then the reaction mixture was refluxed under nitrogen atmosphere at 85 °C for 16 h and at the completion of reaction, the catalyst was removed by filtration through a sintered funnel under suction. The filtrate was evaporated to afford a residue, which was dissolved in ethylacetate (25 mL) and the organic layer was washed with water (3X10 mL), saturate aqueous solution of NaHCO_3 (3X10 mL), water (2X10 mL) and brine (1X10 mL). Drying over anhydrous Na_2SO_4 and evaporation of solvent gave the crude product, which was purified by column chromatography (EtOAc-Hexane - 1:1) to yield **14e** in good yields (931 mg, 58%) as a gum. About 15 % of the unreacted acetoacetate was recovered in the wash. The reaction yield was reproducible upto a scale of 10 mmols.

Procedure B

To a solution of methyl acetoacetate (116 mg, 1 mmol), salicylaldehyde (122 mg, 1 mmol) and acetyl chloride (0.37 mL, 5 mmol) in acetonitrile (10 mL) at ambient temperature was added anhydrous CoCl_2 (6 mg). Then the reaction mixture was refluxed under nitrogen atmosphere at 85 °C for 20 h and at the completion of reaction, the solvent was evaporated under vacuo, and the residue was taken in EtOAc (25 mL) and the organic layer was washed successively with water (2X10 mL), saturated aqueous solution of NaHCO_3 (3X5 mL), water (2X5 mL) and brine (1X5 mL). Drying over anhydrous Na_2SO_4 and evaporation of solvent gave the crude product, which was purified by column chromatography (EtOAc-Hexane - 1:1) to afford the β -keto amide **14e** in good (132 mg, 41 %) yields as a gum. About 18 % of unreacted acetoacetate was recovered. The reaction was reproducible up to a scale of 10 mmols.

^1H NMR, CDCl_3 , δ 7.63 (d, J = 8 Hz, 1H), 7.50 – 7.03 (m, 5H), 6.00 (dd, J = 12.5 Hz & 6Hz, 1H), 4.06 (d, J = 6.9 Hz, 1H), 3.72 (s, 3H), 2.48 (s, 3H), 2.40 (s, 3H), 2.08 (s, 3H);
IR ν_{max} - 3300, 3080, 1750, 1720, 1690 cm^{-1}

Synthesis of Methyl- α -acetyl- β -(*m*-oxyacetyl-*p*-methoxyphenyl)- β -acetamido ester (**14f**)

Procedure A

Polyaniline supported cobalt (II) acetate (~10 mg) was added to a solution of methyl acetoacetate (580 mg, 5 mmol), isovanillin (760 mg, 5 mmol) and acetyl chloride (1.85 mL, 25 mmol), in acetonitrile (25 mL) at ambient temperature. Then the reaction mixture was refluxed under nitrogen atmosphere at 85 °C for 16 h and at the completion of reaction, the catalyst was removed by filtration through a sintered funnel under suction. The filtrate was evaporated to afford a residue. The residue was dissolved in minimum amount of CCl_4 and addition of hexane drop wise to it resulted in precipitation

14g ^1H NMR (CDCl_3) δ 7.33 (d, $J = 7$ Hz, 1H), 6.93 (s, 5H), 5.83 (dd, $J = 8.0, 5$ Hz, 1H), 4.12 (d, $J = 5.0$ Hz, 1H), 3.83 (s, 3H), 3.70 (s, 3H), 2.37 (s, 3H), 2.27 (s, 3H), 2.03 (s, 1.5H), 1.97 (s, 1.5H); IR (KBr) ν_{max} 3330, 3050, 1740, 1720, 1660, 1535, 1340, 1100, 835 cm^{-1} ; MS m/z 369 (M^+), 352, 235, 192, 148.

14g* ^1H NMR, CDCl_3 , δ 7.65 (s, 1H), 7.53 (s, 1H), 7.17 (d, $J = 3$ Hz, 1H), 7.08 (d, $J = 3$ Hz, 1H), 3.87 (s, 1H), 2.48 (s, 6H)

Synthesis of Methyl- α -acetyl- β -(*o*-methylformyl-phenyl)- β -acetamido ester (**14h**)

Procedure A

Polyaniline supported cobalt (II) acetate (~10 mg) was added to a solution of methyl acetoacetate (580 mg, 5 mmol), *o*-methylformylbenzaldehyde (820 mg, 5 mmol) and acetyl chloride (1.1 mL, 15 mmol), in acetonitrile (15 mL) at ambient temperature. Then the reaction mixture was refluxed under nitrogen atmosphere at 85 °C for 12 h and at the completion of reaction, the catalyst was removed by filtration through a sintered funnel under suction. The filtrate was evaporated to afford a residue, which was dissolved in minimum amount of CCl_4 and addition of hexane drop wise to it resulted in precipitation of the crude β -keto amide (**14h**) as a solid. The solid was filtered off on a sintered funnel under suction and the mother liquor was repetitively concentrated and subjected to similar washing with CCl_4 -hexane mixture, until no noticeable amounts of product was left in it (TLC: EtOAc:Hexane - 3:2; $R_f = 0.50$). The combined, crude product was washed once again with CCl_4 and hexane and dried under vacuo to yield **14h** in good yields (683 mg, 51 %) as a solid (M.P. = 145 - 146 °C). About 20 % of the unreacted acetoacetate was recovered in the wash. Addition of additional amounts of acetylchloride or catalyst or increased reaction time did not improve the yields or conversion significantly.

Procedure B

To a solution of methyl acetoacetate (116 mg, 1 mmol), *o*-methylformylbenzaldehyde (164 mg, 1 mmol) and acetyl chloride (0.22 mL, 3 mmol) in acetonitrile (5 mL) at ambient temperature was added anhydrous CoCl_2 (6 mg). Then the reaction mixture was refluxed under nitrogen atmosphere at 85 °C for 14 h and at the completion of reaction the solvent was evaporated under vacuo, and the residue was taken in EtOAc (10 mL) and the organic layer was washed successively with water (1X10 mL), saturated aqueous solution of NaHCO_3 (3X5 mL), water (2X5 mL) and brine (1X5 mL). Drying over anhydrous Na_2SO_4 and evaporation of solvent gave the crude product, which was purified by column chromatography (EtOAc-Hexane - 1:1) or CCl_4 -hexane wash to afford the β -keto amide **14h** in very moderate (41%) yield as a solid. About 20 % of unreacted acetoacetate was recovered. The reaction was reproducible up to a scale of 5 mmols.

^1H NMR (CDCl_3) δ 7.97 (d, $J = 7$ Hz, 1H), 7.66 – 7.30 (m, 4H), 6.42 (dd, $J = 9$ Hz & 3.5 Hz, 1H), 4.13 (d, $J = 3.5$ Hz, 1H), 3.65 (s, 3H), 3.49 (s, 3H), 2.31 (s, 3H), 1.87 (s, 3H); IR (KBr) ν_{max} 3350, 3080, 1740, 1720, 1640 cm^{-1} ; MS m/z 322 (M^+), 206, 189, 164

Synthesis of Methyl- α -acetyl- β -(1-isobutyl)- β -acetamido ester (14i)**Procedure A**

Polyaniline supported cobalt (II) acetate (~10 mg) was added to a solution of methyl acetoacetate (580 mg, 5 mmol) valeraldehyde (130 mg, 5 mmol) and acetyl chloride (1.1 mL, 15 mmol), in acetonitrile (15 mL) at ambient temperature. Then the reaction mixture was refluxed under nitrogen atmosphere at 85 °C for 14 h and at the completion of reaction, the catalyst was removed by filtration through a sintered funnel under suction. The filtrate was evaporated to afford a residue, which was dissolved in ethylacetate (25 mL) and the organic layer was washed successfully with water (2X10 mL), saturated aqueous solution of NaHCO₃ (2X10 mL), water (1X10 mL). Drying (Na₂SO₄) and evaporation of the solvent under vacuo yielded a residue, which was subjected to column chromatography (EtOAc:hexane = 2:3) to yield **14i** (TLC: EtOAc:hexane = 2:3; R_f = 0.5) as a gummy good yields (670 mg, 55%). About 15 % of the unreacted acetoacetate was recovered in the wash. The reaction yield was reproducible up to a scale of 10 mmols.

Procedure B

To a solution of methyl acetoacetate (116 mg, 1 mmol), 3-methylbutanal (86 mg, 1 mmol) and acetyl chloride (0.22 mL, 3 mmol) in acetonitrile (5 mL) at ambient temperature was added anhydrous CoCl₂ (6 mg). Then the reaction mixture was refluxed under nitrogen atmosphere at 85 °C for 14 h and at the completion of reaction, the solvent was evaporated under vacuo, and the residue was taken in EtOAc (10 mL) and the organic layer was washed successively with water (1X10 mL), saturated aqueous solution of NaHCO₃ (3X5 mL), water (2X5 mL) and brine (1X5 mL). Drying over anhydrous Na₂SO₄ and evaporation of solvent gave the crude product, which was purified by column chromatography (EtOAc-Hexane - 2:3) to afford the β -keto amide **14i** in good (127 mg, 52 %) yields as a gum. About 15 % of unreacted acetoacetate was recovered.

¹H NMR, CDCl₃, δ 6.67 (d, J = 10 Hz, 1H), 4.77 (dq, J = 9 Hz & 4.5 Hz, 1H), 3.75 (s, 3H), 2.72 (bs, 1H), 2.30 (s, 3H), 1.93 (s, 3H), 1.47 (dd, J = 8 Hz & 4.8 Hz, 2H), 1.25 (m, 1H), 0.9 (d, J = 6 Hz, 6H); IR ν_{\max} 3280, 3070, 2890, 1775, 1680 cm⁻¹; MS *m/z* 243 (M⁺), 185, 127, 86

Synthesis of 3-methyl-4-acetamido-4-phenyl-butan-2-one (16a)

Polyaniline supported cobalt (II) acetate (~10 mg) was added to a solution of ethylmethylketone (360 mg, 5 mmol), benzaldehyde (530 mg, 5 mmol) and acetyl chloride (1.1 mL, 15 mmol), in acetonitrile (15 mL) at ambient temperature. Then the reaction mixture was refluxed under nitrogen atmosphere at 85 °C for 14 h and at the completion of reaction, the catalyst was removed by filtration through a sintered funnel under suction. The filtrate was evaporated to afford a residue, which was dissolved in minimum amount of CCl₄ and addition of hexane drop wise to it resulted in precipitation of the crude β -keto amide (**16a**) as a solid. The solid was filtered off on a sintered funnel under suction and the mother liquor was repetitively concentrated and subjected to similar washing with CCl₄-hexane mixture, until no noticeable amounts of product was left in it (TLC: EtOAc:Hexane - 1:1; R_f = 0.50). The combined, crude, product was washed once again with CCl₄ and hexane and dried under vacuo to yield the β -keto amide

16a in moderate yields (503 mg, %) as a solid (M.P. = 100 – 102 °C). The reaction yield was reproducible up to a scale of 10 mmols.

^1H NMR, CDCl_3 , δ 8.00 (d, J = 9 Hz, 1H), 7.50 – 7.10 (m, 5H), 5.22 (dd, J = 9 Hz & 9 Hz, 1H), 3.07 (q, J = 7 Hz, 1H), 2.13 (s, 3H), 1.93 (s, 3H), 1.12 (d, J = 7 Hz, 3H); MS m/z 219 (M^+), 176, 148, 106

Synthesis of 3-methyl-4-acetamido-4-(*p*-chlorophenyl)-butan-2-one (**16b**)

Polyaniline supported cobalt (II) acetate (~10 mg) was added to a solution of ethyl methyl ketone (360 mg, 5 mmol), *p*-chlorobenzaldehyde (703 mg, 5 mmol) and acetyl chloride (1.1 mL, 15 mmol), in acetonitrile (15 mL) at ambient temperature. Then the reaction mixture was refluxed under nitrogen atmosphere at 85 °C for 13 h and at the completion of reaction, the catalyst was removed by filtration through a sintered funnel under suction. The filtrate was evaporated to afford a residue. The residue was dissolved in minimum amount of CCl_4 and addition of hexane drop wise to it resulted in precipitation of the crude β -keto amide (**16b**) as a solid. The solid was filtered off on a sintered funnel under suction and the mother liquor was repetitively concentrated and subjected to similar washing with CCl_4 -hexane mixture, until no noticeable amounts of product was left in it (TLC: EtOAc:Hexane - 1:1; R_f = 0.45). The combined, crude product was washed once again with CCl_4 and hexane and dried under vacuo to yield the β -keto amide **16b** in good yields (634 mg, 50 %) as a solid (M.P. = 83 – 85 °C). Addition of additional amounts of acetylchloride or catalyst or increased reaction time did not improve the yields or conversion significantly. The reaction yield was reproducible up to a scale of 10 mmols.

^1H NMR, CDCl_3 , δ 7.36 – 7.03 (m, 4H), 6.97 (d, J = 7.5 Hz, 1H), 5.07 (dd, J = 8.8 Hz & 5.6 Hz, 1H), 3.06 (dq, J = 5 Hz & 1.3 Hz, 1H), 2.00 (s, 3H), 1.96 (s, 3H), 1.13 (d, J = 6.25 Hz, 3H); MS m/z 253 (M^+), 210, 182, 140

Synthesis of 3-methyl-4-acetamido-4-(*m*-methoxy-*p*-oxyacetylphenyl)-butan-2-one (**16c**)

Polyaniline supported cobalt (II) acetate (~10 mg) was added to a solution of methyl acetoacetate (360 mg, 5 mmol), vanillin (760, mg, 5 mmol) and acetyl chloride (1.85 mL, 25 mmol), in acetonitrile (25 mL) at ambient temperature. Then the reaction mixture was refluxed under nitrogen atmosphere at 85 °C for 15 h and at the completion of reaction, the catalyst was removed by filtration through a sintered funnel under suction. The filtrate was evaporated to afford a residue, which was dissolved in minimum amount of CCl_4 and addition of hexane drop wise to it resulted in precipitation of the crude β -keto amide (**16c**) as a solid. The solid was filtered off on a sintered funnel under suction and the mother liquor was repetitively concentrated and subjected to similar washing with CCl_4 -hexane mixture, until no noticeable amounts of product was left in it (TLC: EtOAc:Hexane - 3:2; R_f = 0.40). The combined, crude, product was washed once again with CCl_4 and hexane and dried under vacuo to yield the β -keto amide **16c** in moderate yields (600 mg, 39 %) as a solid (M.P. = 133 – 135°C). Addition of additional amounts of acetylchloride or catalyst or increased reaction time did not improve the yields or conversion significantly.

^1H NMR, CDCl_3 , δ 7.40 (d, J = 5 Hz, 1H), 6.98 – 6.79 (m, 3H), 5.70 (dd, J = 8 Hz & 5 Hz, 1H), 3.73 (s, 3H), 3.09 (d, J = 7 Hz, 1H), 2.17 (s, 3H), 1.77 (s, 3H), 1.35 (s, 3H)

Synthesis of 4-methyl-5-acetamido-5-phenyl-pentan-2-one (16d)

Polyaniline supported cobalt (II) acetate (~10 mg) was added to a solution of methyl acetoacetate (258 mg, 3 mmol), benzaldehyde (318 mg, 3 mmol) and acetyl chloride (0.7 mL, 9 mmol), in acetonitrile (10 mL) at ambient temperature. Then the reaction mixture was refluxed under nitrogen atmosphere at 85 °C for 16 h and at the completion of reaction, the catalyst was removed by filtration through a sintered funnel under suction. The filtrate was evaporated to afford a residue, which was dissolved in minimum amount of CCl_4 and addition of hexane drop wise to it resulted in precipitation of the crude β -keto amide (**16d**) as a solid. The solid was filtered off on a sintered funnel under suction and the mother liquor was repetitively concentrated and subjected to similar washing with CCl_4 -hexane mixture, until no noticeable amounts of product was left in it (TLC: EtOAc:Hexane - 2:3; R_f = 0.50). The combined, crude, product was washed once again with CCl_4 and hexane and dried under vacuo to yield the β -keto amide **16d** in good yields (433 mg, 62 %) as a solid (M.P. = 105 – 107°C).

16d ^1H NMR, CDCl_3 , δ 7.8 (bs, 1H), 7.63 – 7.37 (s, 5H), 5.27 (dd, J = 10 Hz & 6 Hz, 1H), 3.18 (dq, J = 9.4 Hz & 0.8 Hz, 1H), 2.33 (q, J = 7.5 Hz, 2H), 2.04 (s, 3H), 1.26 (d, J = 6.8 Hz, 3H), 1.3 (t, J = 6.8 Hz, 3H); MS m/z 233 (M^+), 189, 147, 133, 105; IR (KBr) ν_{max} 3310, 3060, 1715, 1695, 1640 cm^{-1}

16d* ^1H NMR, CDCl_3 , δ 7.72 – 7.40 (m, 6 H), 3.52 (tdd, J = 26 Hz, 17 Hz & 1.5 Hz, 2H), 2.16 (s, 3H), 1.15 (t, J = 7 Hz, 3H)

Synthesis of α -methyl- β -acetamido- β -phenyl-propiophenone (16e)

Polyaniline supported cobalt (II) acetate (~5 mg) was added to a solution of propiophenone (670 mg, 5 mmol), benzaldehyde (530 mg, 5 mmol) and acetyl chloride (1.17 mL, 15 mmol), in acetonitrile (15 mL) at ambient temperature. Then the reaction mixture was refluxed under nitrogen atmosphere at 85 °C for 16 h and at the completion of reaction, the catalyst was removed by filtration through a sintered funnel under suction. The filtrate was evaporated to afford a residue. The residue was dissolved in minimum amount of CCl_4 and addition of hexane drop wise to it resulted in precipitation of the crude β -keto amide (**16e**) as a solid. The solid was filtered off on a sintered funnel under suction and the mother liquor was repetitively concentrated and subjected to similar washing with CCl_4 -hexane mixture, until no noticeable amounts of product was left in it (TLC: EtOAc:Hexane - 1:1; R_f = 0.5). The combined, crude, product was washed once again with CCl_4 and hexane and dried under vacuo to yield **16e** in moderate yields (41 %) as a solid (M.P. = 130-132 °C). About 25 % of the unreacted acetoacetate was recovered in the wash. Addition of additional amounts of acetylchloride or catalyst or increased reaction time did not improve the yields or conversion significantly. The reaction yield was reproducible up to a scale of 8 mmols.

^1H NMR, CDCl_3 , δ 7.87 – 7.17 (m, 1H), 5.44 (dd, J = 9.4 Hz & 4.4 Hz, 1H), 4.14 (dq, J = 7.5 Hz & 5 Hz, 1H), 1.13 (s, 3H), 1.38 (d, J = 7.5 Hz, 3H); IR (KBr) ν_{max} - 3050, 1750, 1675, 1650 cm^{-1}

Synthesis of α -methyl- β -acetamido- β -(*p*-chlorophenyl)-propiophenone (16f)

Polyaniline supported cobalt (II) acetate (~5 mg) was added to a solution of propiophenone (670 mg, 5 mmol), *p*-chlorobenzaldehyde (700 mg, 5 mmol) and acetyl chloride (1.17 mL, 15 mmol), in acetonitrile (15 mL) at ambient temperature. Then the reaction mixture was refluxed under nitrogen atmosphere at 85 °C for 15 h and at the completion of reaction, the catalyst was removed by filtration through a sintered funnel under suction. The filtrate was evaporated to afford a residue, which was dissolved in ethylacetate (25 mL) and the organic layer was washed successfully with water (2X10 mL), saturated aqueous solution of NaHCO₃ (2X10 mL), water (1X10 mL) and brine (1X10 mL). Drying (Na₂SO₄) and evaporation of the solvent under vacuo yielded a residue, which was subjected to column chromatography (EtOAc: hexane = 2:3) to yield **16f** (TLC: EtOAc:hexane = 4:5; R_f = 0.5) as a gum in moderate yields (653 mg, 42 %). About 22 % of the unreacted propiophenone was recovered in the wash. The aldol product **16f*** was formed in small amounts (~8 %) in the reaction as a side product. The reaction yield was reproducible up to a scale of 10 mmols.

16f ¹H NMR, CDCl₃, δ 7.59 – 7.28 (m, 5H), 7.22 – 7.07 (m, 5H), 5.3 (d, J = 10 Hz, 1H), 4.15 – 3.88 (m, 1H), 2.03 (s, 3H), 1.18 (d, J = 7.5 Hz, 3 H); IR (KBr): ν_{\max} 3260, 3050, 1660, 1630 cm⁻¹.

16f* ¹H NMR, CDCl₃, δ 8.00 – 7.78 (m, 3H), 7.44 – 7.34 (m, 3H), 7.30 – 7.25 (m, 3H), 4.90 (d, J = 7.5 Hz, 1H), 3.69 (dq, J = 7.5 Hz & 3.8 Hz, 1H), 1.02 (d, J = 7.5 Hz, 3H).

Synthesis of α -methyl- β -acetamido- β -(*m*-oxyacetyl-*p*-methoxyphenyl)-propio phenone (16g)**Procedure A**

Polyaniline supported cobalt (II) acetate (~5 mg) was added to a solution of propiophenone (670 mg, 5 mmol), isovanillin (760 mg, 5 mmol) and acetyl chloride (1.95 mL, 25 mmol), in acetonitrile (25 mL) at ambient temperature. Then the reaction mixture was refluxed under nitrogen atmosphere at 85 °C for 16 h and at the completion of reaction, the catalyst was removed by filtration through a sintered funnel under suction. The filtrate was evaporated to afford a residue. The residue was dissolved in minimum amount of CCl₄ and addition of hexane drop wise to it resulted in precipitation of the crude β -keto amide (**16g**) as a solid. The solid was filtered off on a sintered funnel under suction and the mother liquor was repetitively concentrated and subjected to similar washing with CCl₄-hexane mixture, until no noticeable amounts of product (TLC: EtOAc:Hexane - 3:2; R_f = 0.4) was left in it. The combined, crude, product was washed once again with CCl₄ and hexane and dried under vacuo to yield **16g** in good yields (54 %) as a solid (M.P. = 161-163 °C). About 15 % of the unreacted ketone was recovered in the wash. The reaction yield was reproducible up to a scale of 15 mmols.

Procedure B

To a solution of propiophenone (134 mg, 1 mmol), isovanillin (152 mg, 1 mmol) and acetyl chloride (0.37 mL, 5 mmol) in acetonitrile (7.5 mL) at ambient temperature was added anhydrous CoCl₂ (6 mg). Then the reaction mixture was refluxed under nitrogen atmosphere at 85 °C for 18 h. At completion of the reaction, the solvent was evaporated under vacuo, and the residue was taken in EtOAc (10 mL). The organic layer was washed

successively with water (1X10 mL), saturated aqueous solution of NaHCO_3 (3X5 mL), water (2X5 mL) and brine (1X5 mL). Drying over anhydrous Na_2SO_4 and evaporation of solvent gave the crude product, which was purified by column chromatography (EtOAc-Hexane - 2:3) to afford the β -keto amide **16g** in good (47 %) yields as a solid. About 25 % of unreacted ketone was recovered.

^1H NMR (CDCl_3) α 6.50 (d, J = 7.5 Hz, 1H), 4.83 (d.t, J = 11, 5 Hz, 1H), 3.93 (s, 3H), 3.87 (d, J = 3.5 Hz, 1H), 2.53 (s, 1.5H), 2.45 (s, 1.5H), 2.15 (s, 3H), 1.58 (dt, J = 10, 5 Hz, 2H), 1.3 (m, 1H), 1.13 (d, J = 5 Hz, 3H); IR (KBr) ν_{max} 3290, 1750, 1675, 1340, 1100, 850 cm^{-1} ; MS m/z 243 (M^+) 185, 142, 126, 112.

Synthesis of α -methyl- β -acetamido- β -(*p*-oxyacetyl-*m*-methoxyphenyl)-propio phenone (**16h**)

Polyaniline supported cobalt (II) acetate (~5 mg) was added to a solution of propiophenone (670 mg, 5 mmol), vanillin (760 mg, 5 mmol) and acetyl chloride (1.95 mL, 25 mmol), in acetonitrile (25 mL) at ambient temperature. Then the reaction mixture was refluxed under nitrogen atmosphere at 85 °C for 16 h and at the completion of reaction, the catalyst was removed by filtration through a sintered funnel under suction. The filtrate was evaporated to afford a residue. The residue was dissolved in minimum amount of CCl_4 and addition of hexane drop wise to it resulted in precipitation of the crude β -keto amide (**16h**) as a solid. The solid was filtered off on a sintered funnel under suction and the mother liquor was repetitively concentrated and subjected to similar washing with CCl_4 -hexane mixture, until no noticeable amounts of product (TLC: EtOAc:Hexane - 3:2; R_f = 0.45) was left in it. The combined, crude, product was washed once again with CCl_4 and hexane and dried under vacuo to yield **16h** in good yields (48 %) as a solid (M.P. = 142-144 °C). About 20 % of the unreacted ketone was recovered in the wash.

16h ^1H NMR (CDCl_3) α 7.23 (d, J = 4 Hz, 1H), 7.2 (m, 5H), 6.97-6.73 (m, 3H), 5.62 (dd, J = 7.5 Hz, 1H), 4.12 (dq, J = 10, 7 Hz, 1H), 3.68 (s, 3H), 2.16 (s, 3H), 1.94 (s, 3H), 1.31 (d, J = 4Hz, 3H); IR (KBr) ν_{max} 3300, 3030, 1750, 1675, 1305, 1109 cm^{-1} ; MS m/z 369 (M^+), 237, 132.

16h* ^1H NMR, CDCl_3 , δ 9.9 (s, 1H), 7.45 (d, J = 2.5 Hz, 1H), 7.38 (d, J = 2.5 Hz, 1H), 7.22 (s, 1H), 3.83 (s, 3H), 2.23 (s, 3H).

Synthesis of α -methyl- β -acetamido- β -(*p*-nitrophenyl)-propiophenone (**16i**)

Polyaniline supported cobalt (II) acetate (~5 mg) was added to a solution of propiophenone (670 mg, 5 mmol), *p*-nitrobenzaldehyde (755 mg, 5 mmol) and acetyl chloride (15 mmol), in acetonitrile (15 mL) at ambient temperature. Then the reaction mixture was refluxed under nitrogen atmosphere at 85 °C for 16 h and at the completion of reaction, the catalyst was removed by filtration through a sintered funnel under suction. The filtrate was evaporated to afford a residue, which was dissolved in ethylacetate (25 mL) and the organic layer was washed successfully with water (2X10 mL), saturated aqueous solution of NaHCO_3 (2X10 mL), water (1X10 mL) and brine (1X10 mL). Drying (Na_2SO_4) and evaporation of the solvent under vacuo yielded a residue, which was subjected to column chromatography (EtOAc:hexane = 2:3) to yield

16i (TLC: EtOAc:hexane = 2:3; R_f = 0.45) as a gum in moderate yields (587 mg, 36 %). The knoevenegal product was formed as a side product in small amounts (11 %).

^1H NMR, CDCl_3 , δ 8.00 (d, J = 9 Hz, 2H), 7.85 – 7.31 (m, 6H), 7.15 (d, J = 9 Hz, 2H), 5.35 (dd, J = 9 Hz & 6 Hz, 1H), 4.04 (dq, J = 7.2 & 4 Hz, 1H), 2.10 (s, 3H), 1.40 (d, J = 6.6 Hz, 3H); IR ν_{max} 3290, 1675, 1635, 1535, 1340 cm^{-1}

Methyl levulinate

^1H NMR, CDCl_3 , δ 3.86 (s, 3H), 2.68 (dd, J = 10.8 Hz & 7.2 Hz, 4H), 2.23 (s, 3H).

Synthesis of methyl- β -acetyl- γ -acetamido- γ -phenyl-butanoate (**16j**) and methyl-6-acetamido-6-phenyl-hexanoate-4-one (**16k**)

Polyaniline supported cobalt (II) acetate (~5 mg) was added to a solution of methyl levulinate (396 mg, 3 mmol), benzaldehyde (318 mg, 3 mmol) and acetyl chloride (1.17 mL, 15 mmol), in acetonitrile (15 mL) at ambient temperature. Then the reaction mixture was refluxed under nitrogen atmosphere at 85 °C for 18 h and at the completion of reaction, the catalyst was removed by filtration through a sintered funnel under suction. The filtrate was evaporated to afford a residue. The residue was dissolved in minimum amount of CCl_4 and addition of hexane drop wise to it resulted in precipitation of a mixture of two products (TLC : EtOAc:Hexane - 4:5; R_f = 0.4, 0.35) as a gum. The gummy residue was repetitively concentrated and subjected to similar washing with CCl_4 -hexane mixture thrice, and the resulting residue was subjected to column chromatography (Silica gel - EtOAc:Hexane - 3.5:6.5) to yield the β -keto amide **16j** in moderate yields (28 %) as a gum, along with the less substituted regioisomer (**16k**) (18 %) also as a gum.

16j ^1H NMR, CDCl_3 , δ 7.59 – 7.00 (m, 6H), 5.24 (dd, J = 8.8 Hz & 7.8 Hz, 1H), 3.27 (s, 3H), 3.19 (m, 1H), 2.59 (dt, J = 16.25 Hz & 8.8 Hz, 1H), 1.97 (s, 3H), 1.88 (s, 3H); MS m/z 277 (M^+), 176, 148, 106

16k ^1H NMR, CDCl_3 , δ 7.7 (d, J = 9 Hz, 1H), 7.4 – 6.88 (m, 5H), 5.22 (t, J = 8 Hz, 1H), 3.52 (s, 3H), 2.55 (dd, J = 17.5 Hz & 7.5 Hz, 2H), 2.16 – 2.00 (m, 2H), 1.87 (s, 3H), 1.8 – 1.53 (m, 1H)

Synthesis of methyl- β -acetyl- γ -acetamido- γ -(*m*-methoxy-*p*-oxyacetyl)-butanoate (**16l**)

Polyaniline supported cobalt (II) acetate (~5 mg) was added to a solution of methyl acetoacetate (5 mmol), *p*-nitrobenzaldehyde (5 mmol) and acetyl chloride (15 mmol), in acetonitrile (15 mL) at ambient temperature. Then the reaction mixture was refluxed under nitrogen atmosphere at 85 °C for 16 h and at the completion of reaction, the catalyst was removed by filtration through a sintered funnel under suction. The filtrate was evaporated to afford a residue, which was taken in EtOAc (15 mL). The organic layer was washed successively with water (1X10 mL), saturated aqueous solution of NaHCO_3 (3X5 mL), water (2X5 mL) and brine (1X5 mL). Drying over anhydrous Na_2SO_4 and evaporation of solvent gave the crude product, which was purified by column chromatography (EtOAc-Hexane - 2:3) to afford the β -keto amide **16l** in

moderate (37 %) yields as a gum. About 25 % of unreacted keto ester was recovered in the column. The knoevenegal product was formed as a side product in copious amounts (~25 %).

^1H NMR, CDCl_3 , δ 7.15 – 6.90 (m, 3H), 5.13 (dd, J = 8.8 Hz & 5 Hz, 1H), 3.69 (s, 3H), 3.38 (m, 1H), 2.58 (td, J = 15 Hz & 5.6 Hz, 2H), 2.16 (s, 3H), 1.98 (s, 3H), 1.81 (s, 3H); IR ν_{max} 3300, 3050, 1750, 1660 cm^{-1}

General Procedure for the synthesis of β -aryl homoisothreonines

Sodium borohydride (1 mmol) was added in portions to a solution of β -acetamido ketone (1 mmol) in methanol (75 mL) with constant stirring at 0-5° C. After complete addition, the reaction was further stirred for 1.5 h. After completion of reaction (TLC), the reaction was quenched with a few drops of saturated aqueous solution of NH_4Cl and the solvent was evaporated in vacuo. The residue was taken in diethyl ether (15 mL), and the organic layer was washed successively with water (2X10 mL) and brine (1X10 mL). Drying (anhydrous Na_2SO_4) and evaporation of solvent gave the crude product, which was purified by column chromatography (silica gel; EtOAc-Hexane) to afford the corresponding alcohols (β -aryl homoisothreonines) in good yields (80 - 90 %).

Synthesis of methyl-N-acetyl- β -phenyl-homoisothreoninate (18a)

Sodium borohydride (38 mg, 1 mmol) was added in portions to a solution of **14a** (263 mg, 1 mmol) in methanol (75 mL) with constant stirring at 0-5° C. After complete addition, the reaction was further stirred for 1.5 h. After completion of reaction (TLC), the reaction was quenched with a few drops of saturated aqueous solution of NH_4Cl and the solvent was evaporated in vacuo. The residue was taken in diethyl ether (15 mL), and the organic layer was washed successively with water (2X10 mL) and brine (1X10 mL). Drying (anhydrous Na_2SO_4) and evaporation of solvent gave the crude product, which was purified by column chromatography (silica gel; EtOAc-Hexane) to afford the corresponding β -aryl homoisothreonine **18a** in good yields (235 mg, 90 %) as gum.

^1H NMR, CDCl_3 , δ 8.00 (d, J = 8 Hz, 1H), 7.35 – 7.08 (s, 5H), 5.61 (dd, J = 11 Hz & 2.5 Hz, 1H), 3.87 (dq, J = 6.8 Hz & 3 Hz, 1H), 3.53 (s, 3H), 2.93 – 2.65 (m, 1H), 1.97 (s, 3H), 1.17 (d, J = 6 Hz, 3H); IR ν_{max} 3380, 3050, 1750, 1650, 1530 cm^{-1} .

Synthesis of methyl-N-acetyl- β -(*p*-chlorophenyl)-homoisothreoninate (18b)

Sodium borohydride (38 mg, 1 mmol) was added in portions to a solution of **14b** (300 mg, 1 mmol) in methanol (75 mL) with constant stirring at 0-5° C. After complete addition, the reaction was further stirred for 1.5 h. After completion of reaction (TLC), the reaction was quenched with a few drops of saturated aqueous solution of NH_4Cl and the solvent was evaporated in vacuo. The residue was taken in diethyl ether (15 mL), and the organic layer was washed successively with water (2X10 mL) and brine (1X10 mL). Drying (anhydrous Na_2SO_4) and evaporation of solvent gave the crude product, which was purified by column chromatography (silica gel; EtOAc-Hexane) to afford the corresponding β -aryl homoisothreonine **18b** in good yields (270 mg, 90 %) as a gum.

^1H NMR (CDCl_3) δ 7.25(m, 4H), 7.16 (d, J = 6 Hz, 1H), 5.63 (dd, J = 10, 3 Hz, 1H), 4.15 (m, 1H), 3.55 (s, 3H), 2.89 (dd, J = 11.3 Hz, 1H), 2.23 (s, 1.5H), 2.08 (s, 1.5H), 1.26 (d, J = 6 Hz, 3H); IR (KBr) ν_{max} 3350, 3050, 1750, 1655, 1530, 1425 cm^{-1} .

Synthesis of methyl-N-acetyl- β -(*p*-nitrophenyl)-homoisothreoninate (18c)

Sodium borohydride (38 mg, 1 mmol) was added in portions to a solution of 14c (308 mg, 1 mmol) in methanol (75 mL) with constant stirring at 0-5° C. After complete addition, the reaction was further stirred for 1.5 h. After completion of reaction (TLC), the reaction was quenched with a few drops of saturated aqueous solution of NH_4Cl and the solvent was evaporated in vacuo. The residue was taken in diethyl ether (15 mL), and the organic layer was washed successively with water (2X10 mL) and brine (1X10 mL). Drying (anhydrous Na_2SO_4) and evaporation of solvent gave the crude product, which was purified by column chromatography (silica gel; EtOAc-Hexane) to afford the corresponding β -aryl homoisothreonine 18c in good yields (262 mg, 85 %) as gum.

^1H NMR, CDCl_3 , δ , 8.17 (d, J = 9 Hz, 2 H), 7.48 (d, J = 9 Hz, 2H), 7.38 (d, J = 8.8 Hz, 1H), 5.63 (dd, J = 8 Hz & 5.5 Hz, 1H), 3.85 (dq, J = 8 Hz & 2.5 Hz, 1H), 3.65 (s, 3H), 2.80 (dd, J = 6.25 Hz & 3 Hz, 1H), 2.06 (s, 3H), 1.28 (d, J = 6.5 Hz, 3H); IR ν_{max} 3380, 3050, 1740, 1715, 1675, 1530 cm^{-1}

Synthesis of methyl-N-acetyl- β -(*o*-methylformyl-phenyl)-homoisothreoninate (18d)

Sodium borohydride (38 mg, 1 mmol) was added in portions to a solution of 14h (320 mg, 1 mmol) in methanol (75 mL) with constant stirring at 0-5° C. After complete addition, the reaction was further stirred for 1.5 h. After completion of reaction (TLC), the reaction was quenched with a few drops of saturated aqueous solution of NH_4Cl and the solvent was evaporated in vacuo. The residue was taken in diethyl ether (15 mL), and the organic layer was washed successively with water (2X10 mL) and brine (1X10 mL). Drying (anhydrous Na_2SO_4) and evaporation of solvent gave the crude product, which was purified by column chromatography (silica gel; EtOAc-Hexane) to afford the corresponding β -aryl homoisothreonine 18d in good yields (255 mg, 80 %) as a gum.

^1H NMR, CDCl_3 , δ 7.38 – 7.16 (m, 4H), 6.89 (d, J = 8 Hz, 1H), 5.62 (dd, J = 8 Hz & 4.5 Hz, 1H), 3.98 (m, 1H), 3.65 (s, 3H), 3.51 (s, 3H), 2.69 (dd, J = 10.2 Hz & 4.2 Hz, 1H), 2.00 (s, 3H), 1.33 (d, J = 6.6 Hz, 3H); IR ν_{max} 3400, 3280, 1720, 1650, 1535 cm^{-1}

Synthesis of methyl-N-acetyl- β -(1-isobutyl)-homoisothreoninate (18e)

Sodium borohydride (38 mg, 1 mmol) was added in portions to a solution of 14i (243 mg, 1 mmol) in methanol (75 mL) with constant stirring at 0-5° C. After complete addition, the reaction was further stirred for 1.5 h. After completion of reaction (TLC), the reaction was quenched with a few drops of saturated aqueous solution of NH_4Cl and the solvent was evaporated in vacuo. The residue was taken in diethyl ether (15 mL), and the organic layer was washed successively with water (2X10 mL) and brine (1X10 mL). Drying (anhydrous Na_2SO_4) and evaporation of solvent gave the crude product, which was

purified by column chromatography (silica gel; EtOAc-Hexane) to afford the corresponding β -aryl homoisothreonine **18e** in good yields (200 mg, 82 %) as a gum.

^1H NMR, CDCl_3 , δ 7.25 (d, J = 10 Hz, 1H), 6.83 (dt, J = 10 Hz & 3.6 Hz, 1H), 4.20 – 3.87 (m, 1H), 3.70 (s, 3H), 2.6 (dd, J = 13 Hz & 9 Hz, 1H), 2.0 (s, 3H), 1.27 (d, J = 6 Hz, 3H), 1.33 – 1.02 (m, 3H), 0.88 (d, J = 6 Hz, 6H); IR ν_{max} 3390, 1720, 1695, 1640.

Synthesis of 2-hydroxy-3-methylformyl-4-acetamido-4-phenyl-butane (**18f**)

Sodium borohydride (38 mg, 1 mmol) was added in portions to a solution of **16a** (219 mg, 1 mmol) in methanol (75 mL) with constant stirring at 0-5° C. After complete addition, the reaction was further stirred for 1.5 h. After completion of reaction (TLC), the reaction was quenched with a few drops of saturated aqueous solution of NH_4Cl and the solvent was evaporated in vacuo. The residue was taken in diethyl ether (15 mL), and the organic layer was washed successively with water (2X10 mL) and brine (1X10 mL). Drying (anhydrous Na_2SO_4) and evaporation of solvent gave the crude product, which was purified by column chromatography (silica gel; EtOAc-Hexane) to afford the corresponding β -aryl homoisothreonine **18f** in good yields (80 %).

^1H NMR, CDCl_3 , δ 7.58 (d, J = 9 Hz, 1H), 7.37 – 7.05 (s, 5H), 4.69 (dd, J = 9 Hz & 7.5 Hz, 1H), 3.82 (dq, J = 15 Hz & 6 Hz, 1H), 2.44 (s, J = 7.5 Hz, 1H), 1.92 (s, 3H), 1.09 (d, J = 9.5 Hz, 3H), 0.73 (d, J = 7.5 Hz, 3H)

Synthesis of 3-(1-acetamido-1-benzyl)-4-methyl-g-lactone (**18g**)

Sodium borohydride (38 mg, 1 mmol) was added in portions to a solution of **16j** (275 mg, 1 mmol) in methanol (75 mL) with constant stirring at 0-5° C. After complete addition, the reaction was further stirred for 1.5 h. After completion of reaction (TLC), the reaction was quenched with a few drops of saturated aqueous solution of NH_4Cl and the solvent was evaporated in vacuo. The residue was taken in diethyl ether (15 mL), and the organic layer was washed successively with water (2X10 mL) and brine (1X10 mL). Drying (anhydrous Na_2SO_4) and evaporation of solvent gave the crude product, which was purified by column chromatography (silica gel; EtOAc-Hexane) to afford the corresponding β -aryl homoisothreonine derivative lactone **18g** in moderate yields (180 mg, 66%) as a gum.

^1H NMR, CDCl_3 , δ 7.55 (d, J = 7.5 Hz, 1H), 7.41 – 7.07 (m, 5H), 5.39 (dd, J = 13.5 Hz & 6 Hz, 1H), 4.08 (q, J = 7.25 Hz, 1H), 3.55 (d, J = 10 Hz, 2H), 2.91 (dd, J = 6.3 Hz & 2 Hz, 1H), 2.03 (s, 3H), 1.22 (d, J = 7 Hz, 3H); IR ν_{max} 3290, 1790, 1765, 1735, 1370 cm^{-1}

Synthesis of β -phenyl-N-acetyl-homoisothreonine (**19a**)

To a solution of **14a** (263 mg, 1 mmol) in methanol (mL) was added a solution of KOH (57 mg, 1 mmol) in warm water (1 mL) and stirred for 4 h after which, an additional amount of KOH (28 mg, 0.5 mmol) was added and the reaction mixture was stirred for further 1h. The solvent was removed under vacuo and to the resulting residue was added dichloromethane (mL), followed by drop wise addition of 1N HCl while. After complete acidification of the lithium salt, the organic layer was separated, dried and concentrated

under vacuo to yield the carboxylic acid **19a** as a hygroscopic deliquescent solid in moderate yields (105 mg, 42%).

^1H NMR, CDCl_3 , δ 8.00 (bs, 1H), 7.22 (s, 5 H), 5.56 (dd, $J = 15$ Hz & 8.8 Hz, 1H), 3.95 (m, 1H), 2.83 (dd, $J = 10$ Hz & 4.3 Hz, 1H), 1.99 (s, 3H), 1.25 (d, $J = 6.3$ Hz, 3H).

Synthesis of methyl- β -phenyl-homoisothreoninate hydrochloride (**19b**)

To **14a** (263 mg, 1 mmol) in a dry flask was added a 2N solution of HCl in methanol (6 mL) and the contents of the flask were refluxed for 8h. The solvent was removed under vacuo and the resulting residue was washed with ether and dichloromethane. Drying of the resulting residue yielded **19b** as a white hygroscopic solid (M.P. 62°C) in good yields (185 mg, 72%).

^1H NMR, CDCl_3 :DMSO- d_6 , δ 7.45 – 7.03 (m, 5H), 4.48 (bs, 2H), 3.95 (dq, $J = 10.6$ Hz & 5.6 Hz, 1H), 3.62 (m, 1H), 3.59 (s, 3H), 2.96 (bs, 1H), 2.8 (dd, $J = 10.6$ Hz & 4.4 Hz, 1H), 1.22 (d, $J = 6.9$ Hz, 3H); IR ν_{max}

Synthesis of β -phenyl-homoisothreonine (**19c**)

To **14a** (263 mg, 1 mmol) in a dry flask was added a 80% solution (v/v) of HCl in methanol (10 mL) and the contents of the flask were refluxed for 12 h. The solvent was removed under vacuo and the resulting residue was washed with ether and dichloromethane. The resulting solid was dried on a cellulose filter paper under vacuum until constant mass, to yield β -phenyl-homoisothreonine hydrochloride as a white solid (M.P. = 86 °C) in good yields (155 mg, 65 %). To a suspension of the resulting solid in methanol was added triethylamine (mL, mmol) drop wise and the contents were stirred for 15 min. Removal of solvent and washing of the resulting residue with water and drying in a vacuum dessicator for 10 h yielded the β -amino acid **19c** as a white solid (M.P. = 156 – 158 °C) in good yields (93 mg, 60%).

^1H NMR, CDCl_3 :DMSO- d_6 , δ 7.35 – 6.96 (m, 5H), 5.98 (bd, $J = 8.8$ Hz, 2H), 3.89 (dq, $J = 10.6$ Hz & 5.6 Hz, 1H), 2.98 (m, 1H), 2.79 (m, 1H), 1.22 (d, $J = 6.25$ Hz, 3H).

Synthesis of methyl-N-(methyloxycarbonyl)- β -phenyl-homoisothreoninate (**20a**)

To an ice-cold solution of **19b** (100 mg, 0.39 mmol) in THF (5 mL) was added triethylamine (0.12 mL, 0.85 mmol) and stirred for 10 min. To this was added methylchloroformate (38 mg, 0.39 mmol). After stirring for 1 h the contents of the flask were concentrated under vacuo and the resulting residue was taken in ethylacetate (10 mL). The organic solution was washed with 1N HCl (1X10 mL), water (2X10 mL) and brine (1X 10mL). The organic layer was dried (Na_2SO_4) and concentrated under vacuo and subjected to column chromatography to yield **20a** as a gum in good yields (89 mg, 82%).

^1H NMR, CDCl_3 , δ 7.33 – 7.11 (m, 5H), 6.49 (d, $J = 8.8$ Hz, 1H), 5.19 (dd, $J = 8$ Hz & 8 Hz, 1H), 4.1 – 3.91 (m, 1H), 3.61 (s, 3H), 3.48 (s, 3H), 2.78 (dd, $J = 8.3$ Hz & 2.5 Hz, 1H), 1.17 (d, $J = 6.6$ Hz, 3H)

Synthesis of methyl-N-(benzoyl)- β -phenyl-homoisothreoninate (20b)

To an ice-cold solution of **19b** (150 mg, 0.58 mmol) in THF (10 mL) was added triethylamine (0.18 mL, 1.28 mmol) and stirred for 10 min. To this was added benzoylchloride (81 mg, 0.58 mmol). After stirring for 2 h the contents of the flask were concentrated under vacuo and the resulting residue was taken in ethylacetate (15 mL). The organic solution was washed with 1N HCl (1X10 mL), saturated solution of NaHCO₃ (2X10 mL), water (2X10 mL) and brine (1X 10mL). The organic layer was dried (Na₂SO₄) and concentrated under vacuo and subjected to column chromatography (silicagel EtOAc:hexane) to yield **20b** as a solid (M.P.= 104 – 106°C) in good yields (135 mg, 71 %).

¹H NMR, CDCl₃, δ 8.00 (dd, J = 7.5 Hz & 6.9 Hz, 1H), 7.83 – 7.6 (m, 2H), 7.53 – 7.14 (m, 8 H), 5.75 (dd, J = 7.5 Hz & 6.25 Hz, 1H), 5.39 (bs, 1H), 3.95 (dq, J = 6.25 Hz & 1.3 Hz, 1H), 3.61 (s, 3H), 2.84 (dd, J = 6.25 Hz & 2.5 Hz, 1H), 1.20 (d, J = 6.1 Hz, 3H); IR (KBr) ν_{\max} 3400, 3280, 3020, 1750, 1650 cm⁻¹

Synthesis of methyl-N-(cinnamoyl)- β -phenyl-homoisothreoninate (20c)

To an ice-cold solution of **19b** (150 mg, 0.58 mmol) in THF (10 mL) was added triethylamine (0.18 mL, 1.28 mmol) and stirred for 10 min. To this was added cinnamoylchloride (96 mg, 0.58 mmol). After stirring for 4 h the contents of the flask were concentrated under vacuo and the resulting residue was taken in ethylacetate (15 mL). The organic solution was washed with 1N HCl (1X10 mL), saturated solution of NaHCO₃ (2X10 mL), water (2X10 mL) and brine (1X10mL). The organic layer was dried (Na₂SO₄) and concentrated under vacuo and subjected to column chromatography (silicagel, EtOAc : hexane) to yield **20c** as a solid (M.P. = 109 – 112 °C) in good yields (147 mg, 72 %).

¹H NMR, CDCl₃, δ 7.61 (d, J = 15 Hz, 1H), 7.42 – 7.1 (m, 11H), 6.52 (d, J = 15 Hz, 1H), 5.58 (dd, J = 11.3 Hz & 6.9 Hz 1H), 4.00 (dq, J = 12.5 Hz & 6.9 Hz, 1H), 3.45 (s, 3H), 2.97 (dd, J = 15 Hz & 7.5 Hz, 1H), 1.16 (d, J = 6.3 Hz, 3H); IR ν_{\max} 3380, 3300, 3220, 2930, 1730, 1660, 1600 cm⁻¹

Synthesis of benzyl-N-acetyl- β -phenyl-homoisothreoninate (24)

To a solution of **14a** (263 mg, 1 mmol) in acetonitrile (5 mL) was added benzylalcohol (540 mg, 5 mmol) and anhydrous CoCl₂ (3 mg) and the contents of the flask were refluxed for 12 h. The solvent was removed under vacuo and the resulting residue was taken in EtOAc and washed with water (2 X 10mL). The organic layer was separated, dried (Na₂SO₄) and concentrated under vacuo and subjected to colmnchromatography to obtain the trans esterified product **24** in moderate yields (176 mg, 52 %) as a solid gum.

¹H NMR, CDCl₃, δ 7.48 – 7.15 (m, 11H), 5.97 – 5.61 (m, 1H), 4.72 (bs, 2H), 4.15 (d, J = 4.5 Hz, 1H), 2.26 (s, 3H), 2.04 (s, 3H); IR ν_{\max} 3200, 3010, 2920, 1760, 1600, 1550.

Synthesis of 2-oxyacetyl-3-methylformyl-4-acetamido-4-phenyl-2-butanol (21)

To a solution of **18a** (150 mg, 0.57 mmol) in DCM (5 mL) was added anhydrous zinc chloride (~ 15 mole %) and stirred for 15 minutes at 0° C, followed by addition of acetic anhydride (87 mg) (freshly distilled). After ½ h, the reaction vessel was allowed to warm to room temperature and stirred for further 6 h. Water (5 mL) was added to it and stirred for further 15 min. after which CH₂Cl₂ (15 mL) was added to it and the aqueous layer was separated. Washing the organic layer with saturated aqueous solution of NaHCO₃ (1X10 mL), water (1X10 mL) and water (1X10 mL); drying (Na₂SO₄) and evaporation of solvent in vacuum yielded a residue, which was subjected to column chromatography (EtOAc : hexane) to yield the acetylated product **21** in good yields (212 mg, 69 %) as a gum.

¹H NMR, CDCl₃, δ 7.50 – 7.08 (s, 5 H), 6.53 (d, J = 8.8 Hz, 1H), 5.45 (dd, J = 8.8 Hz & 8 Hz, 1H), 5.22 (q, J = 4.5 Hz, 1H), 3.5 (s, 3H), 3.11 (dd, J = 10 Hz & 4.4 Hz, 1H), 2.00 (s, 3H), 1.85 (s, 3H), 0.7 (d, J = 6.5 Hz, 3H).

Synthesis of 3-methylformyl-4-acetamido-4-parachlorophenyl-2-butene (23)

To an ice-cold solution of **18b** (100 mg, 0.33 mmol) in CH₂Cl₂ (8 mL) was added pyridine (0.1 mL) followed by mesylchloride (57 mg, 0.5 mmol) and stirred at room temperature for 15 h. The reaction mixture was diluted with CH₂Cl₂ (20 mL) and the organic solution was washed with saturated aqueous solution of NaHCO₃ (2X10 mL), water (1X10 mL) and brine. Drying (Na₂SO₄) and evaporation of organic solvent under vacuo yielded the crude mesylated product **22** which was dissolved in dichloromethane (15 mL) and after addition of triethylamine (0.07 mL, 0.5 mmol) was set to reflux for 18 h. Dilution with CH₂Cl₂ (15 mL) and washing the organic layer with water (2X10 mL) and brine (1X10 mL); drying it (Na₂SO₄) and concentration under vacuo yielded a residue, which was subject to column chromatography (EtOAc : hexane) to yield the required product **23** in good overall yield (38 mg, 47 %) as an oil.

22 ¹H NMR, CDCl₃, δ 7.38 – 7.00 (m, 5H), 5.15 (dd, J = 21 Hz & 9 Hz, 1H), 4.12 (m, 1H), 3.63 (s, 3H), 3.20 (dd, J = 8.8 Hz & 4.5 Hz, 1H), 3.11 (s, 3H), 2.00 (s, 3H), 1.52 (d, J = 7.5 Hz, 3H)

23 ¹H NMR, CDCl₃, δ 7.32 – 6.98 (m, 5H), 5.37 (d, J = 6 Hz, 1H), 4.61 (m, 1H), 3.59 (s, 3H), 2.13 (d, J = 6 Hz, 3H), 1.85 (s, 3H)

Synthesis of compound 26

To a solution of the acetamido ketone **16j** (137 mg, 0.5 mmol) in methanol (4 mL) was added a solution of LiOH.H₂O (20 mg, 0.5 mmol) in water (1 mL) and the reaction mixture was allowed to stir for 4 h at ambient temperature. The solvent was removed and to the resulting residue was added, dilute (1N) HCl until complete acidification of the lithium carboxylate, formed from **16j**. The carboxylic acid was extracted in CH₂Cl₂ (15 mL) and washed with water (2 X5 mL). The organic layer was dried (Na₂SO₄) and concentrated in vacuo to yield the crude corresponding carboxylic acid **25** as a solid (M.P. = 80-82 °C). The carboxylic acid **25** (102 mg, 0.37 mmol) was dissolved in THF (1

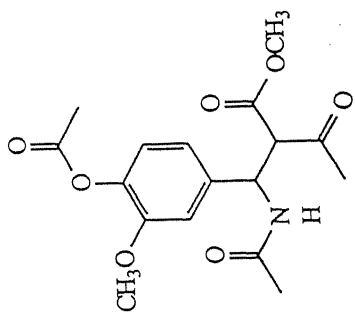
mL) and to it was added triethylamine (0.05 mL, 0.37 mmol) and the reaction vessel was cooled to -5 °C in an ice-salt bath. Methylchloroformate (0.03 mL, 0.37 mmol) was added to it and stirred for 50 - 60 seconds, followed by the addition of a solution of methyl-L-tyrosinate hydrochloride (94 mg, 0.41 mmol) in DMSO (0.5 mL) and a solution of triethylamine (0.06 mL, 0.4 mL) in THF (1 mL). The reaction mixture was stirred for a further 3 h. Triethylamine hydrochloride was filtered off under suction on a sintered funnel and washed with THF. The solvent was evaporated from the filtrate under vacuo and the resulting residue was subjected to column chromatography (Silica gel - EtOAc:Hexane - 2:3) to yield the required peptidomimetic **26** in good yields (58 %) as a gum.

^1H NMR, CDCl_3 , δ 7.52 – 7.18 (m, 5H), 7.15 – 7.02 (m, 3H), 6.98 – 6.72 (m, 3H), 5.42 (dd, $J = 9.6$ Hz & 3 Hz, 1H), 4.72 – 4.4 (m, 1H), 3.22 – 3.17 (m, 1H), 3.00 (d, $J = 6$ Hz, 2H), 2.52 (dd, $J = 12$ Hz & 6 Hz, 1H), 1.96 (s, 3H), 1.87 (s, 3H); IR ν_{max} 3360 (br), 3300 (s), 2920, 1775, 1660, 1435.

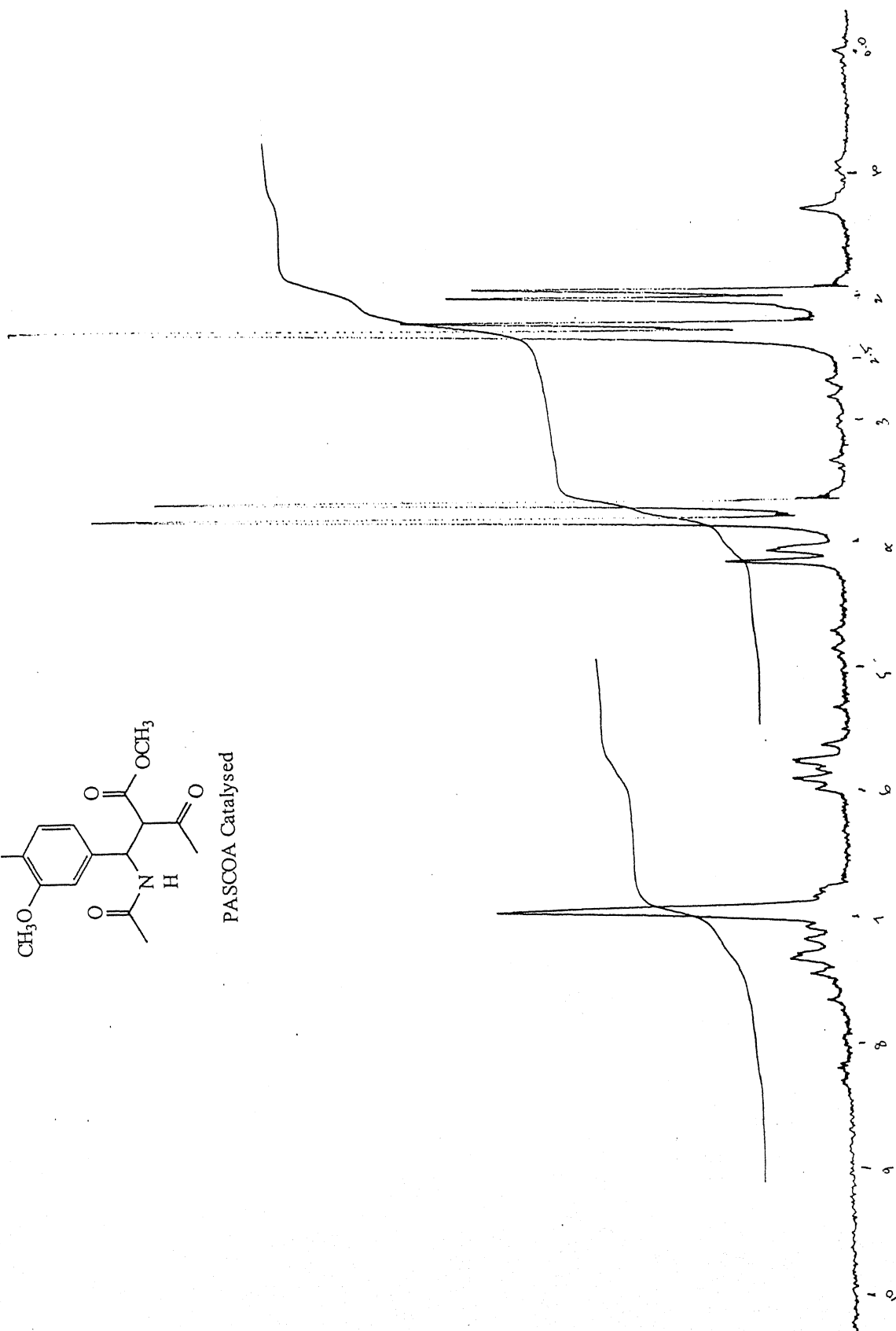
Synthesis of methyl-N-acetyl- β -phenyl-homoisothreonine-L-(4-*trans*-hydroxy)-prolinate (**27**)

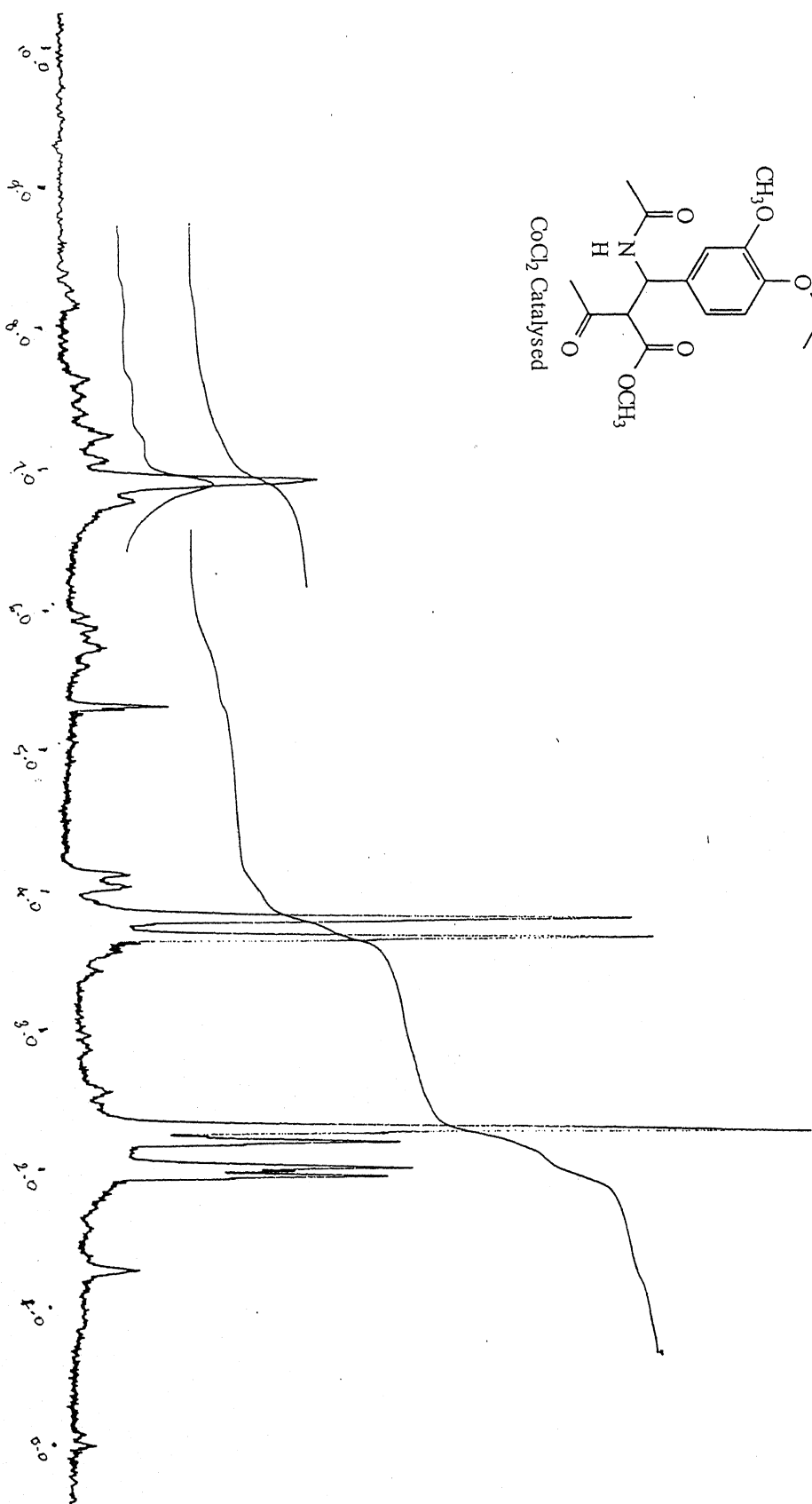
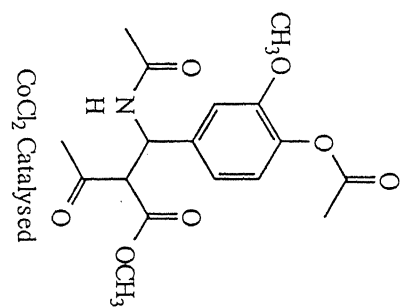
To a solution of **19a** (250 mg, 0.95 mmol) in dry dichloromethane (5 mL) was added triethylamine (0.15 mL, 1.05 mmol) and the reaction vessel was cooled to 0 °C. To this was added 1-hydroxybenzotriazole (192 mg, 1.43 mmol), methyl-L-(4-*trans*-hydroxy)-prolinate hydrochloride (172 mg, 1.43 mmol) and triethylamine (0.22 mL, 1.57 mmol) in succession and allowed to stir for 10 min. Then dicyclohexylcarbodiimide (DCC) (295 mg, 1.43 mmol) was added to it at 0 °C and stirred at that temperature for 10 min. The reaction mixture was warmed to room temperature after 10 min. and stirred for 18 h. The reaction mixture was diluted with dichloromethane (20 mL) and washed with saturated aqueous solution of NaHCO_3 (2X10 mL), water (1X10 mL) and brine (1X10 mL). The organic layer was separated, dried (Na_2SO_4) and concentrated under vacuo and subjected to column chromatography to obtain the dipeptide isostere **27** in good yields (186 mg, 52 %) as a gum.

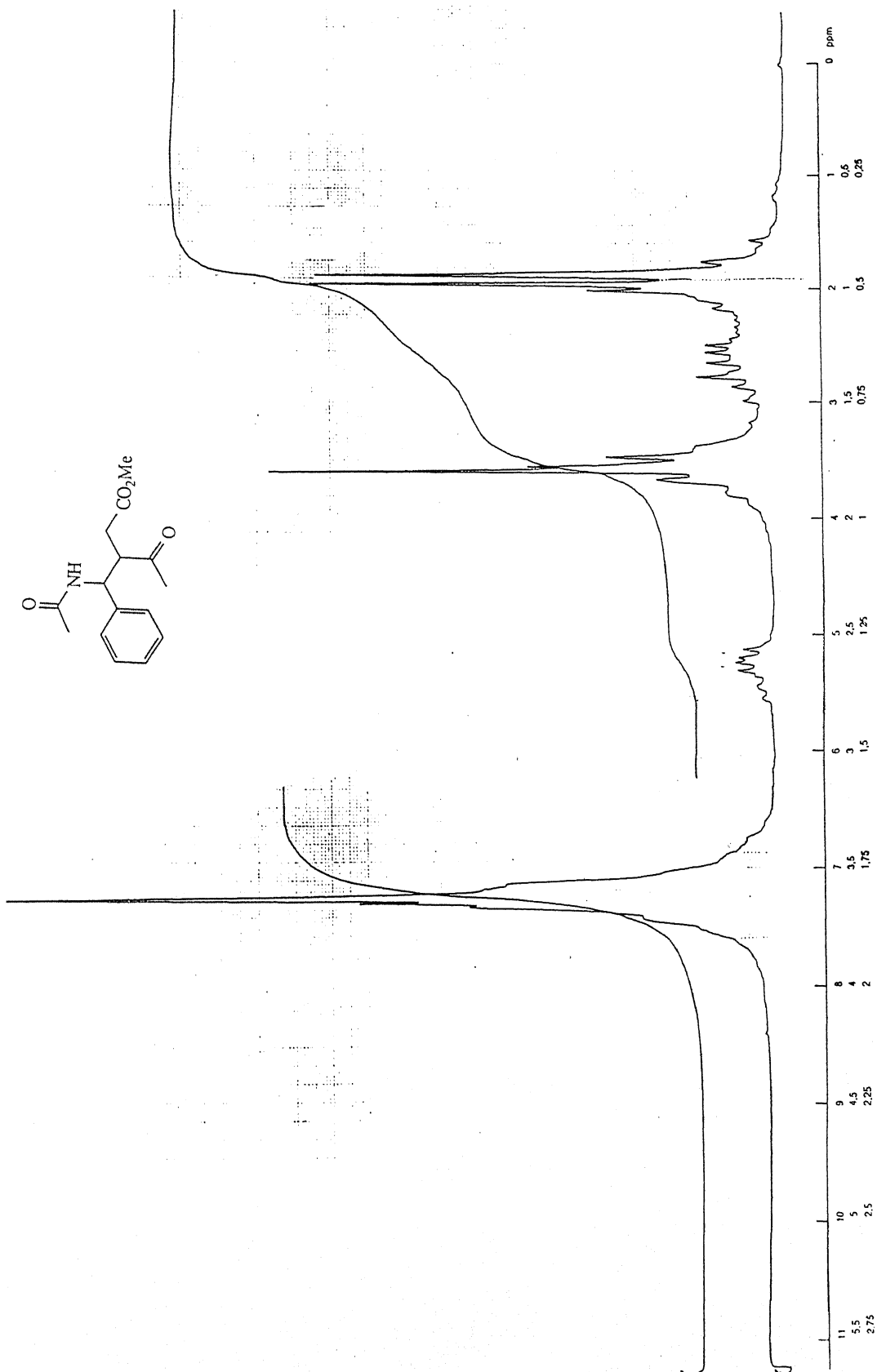
^1H NMR 400 MHz, CDCl_3 , δ 7.58 (d, $J = 7.8$ Hz, 1H), 7.40 - 7.23 (m, 5H), 4.66 - 4.62 (m, 1H), 4.47 (t, $J = 5.2$ Hz, 1H), 4.41 (bs, 1H), 4.20 (bs, 1H), 3.77 (s, 3H), 3.54 (d, $J = 9$ Hz, 1H), 3.48 (t, $J = 4.1$ Hz, 1H), 2.98 (d, $J = 9$ Hz, 1H), 2.08 (s, 3H), 2.00 (d, $J = 6.6$ Hz, 2H), 0.88 (t, $J = 4.8$ Hz, 3H); IR ν_{max} 3400 - 3300(br), 3330, 2980, 1775, 1665, 1550 cm^{-1}

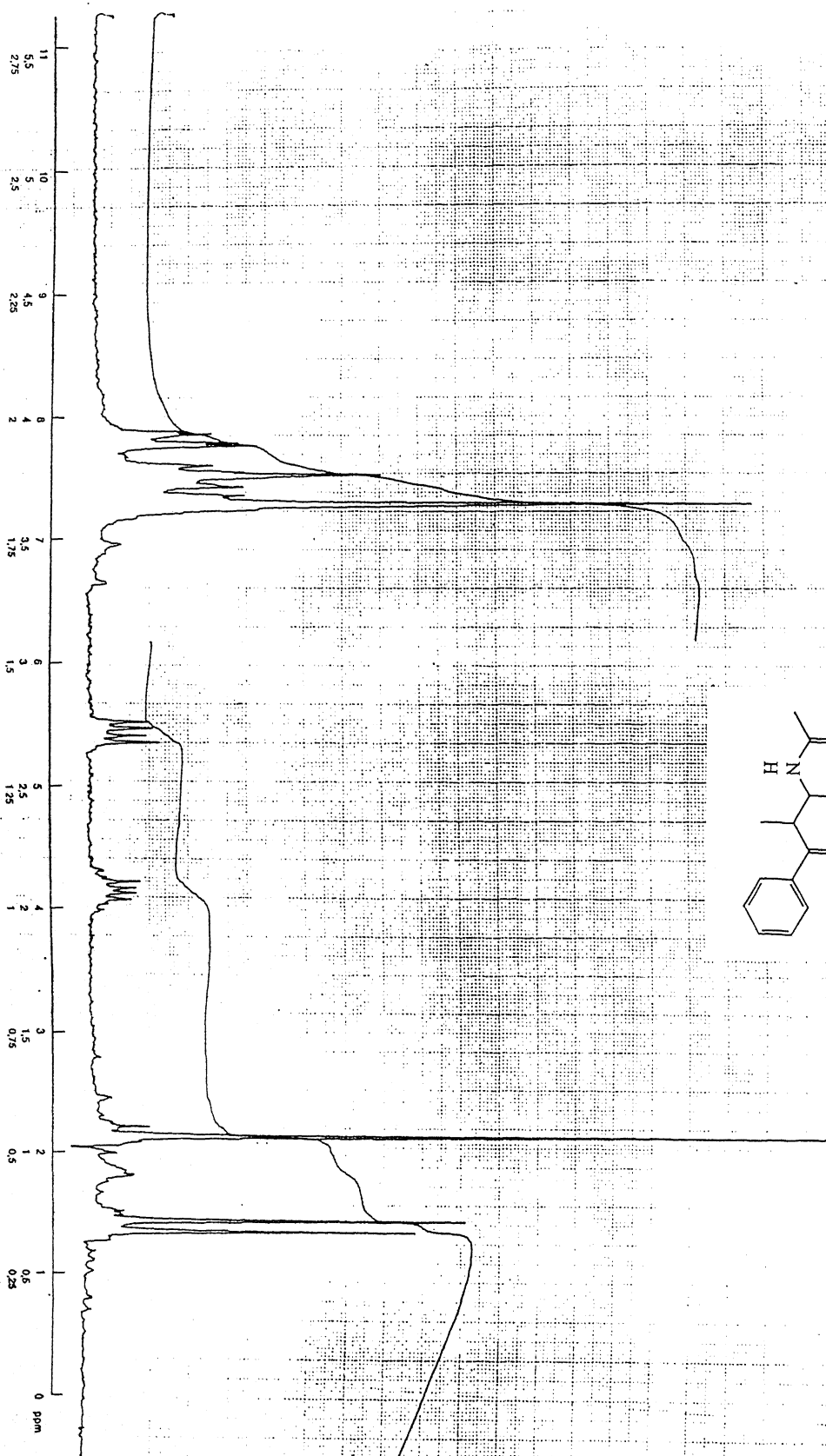
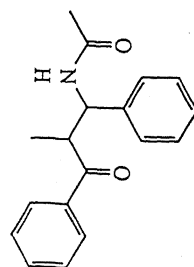


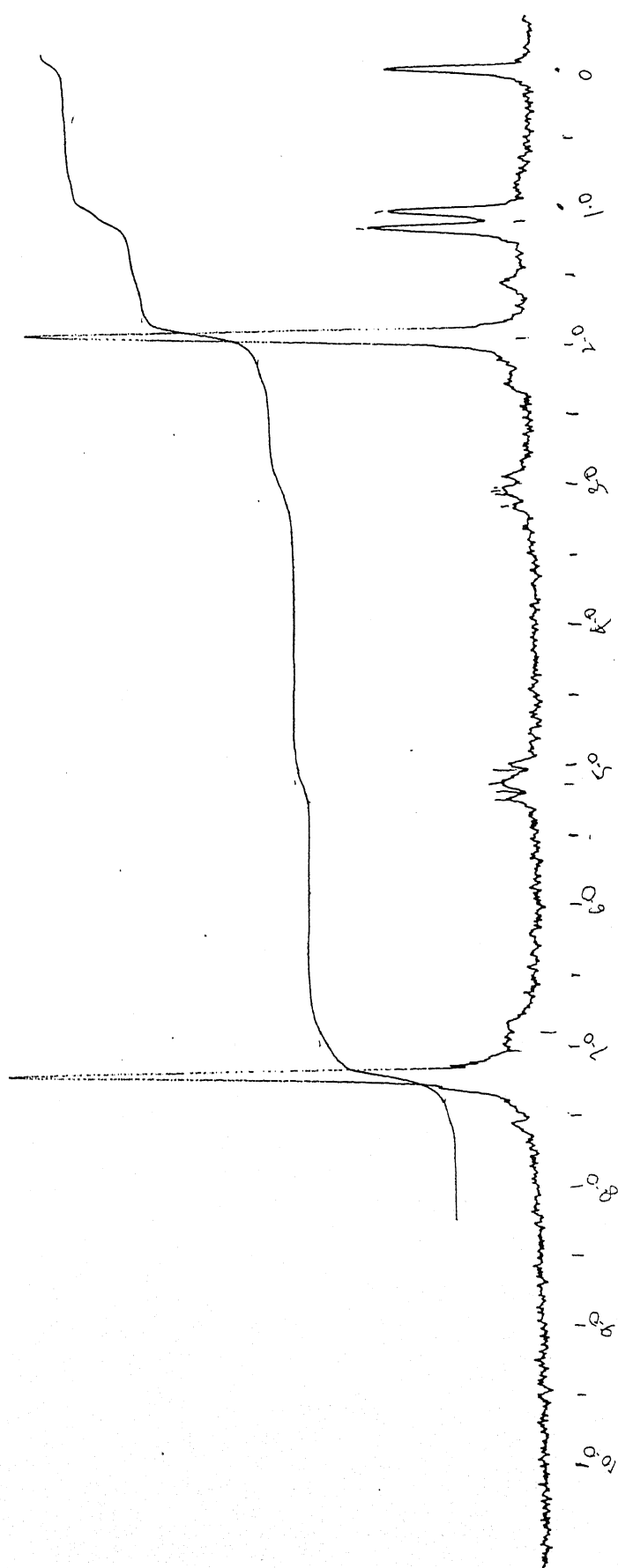
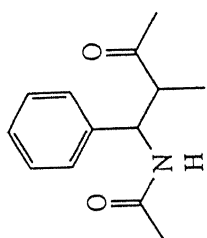
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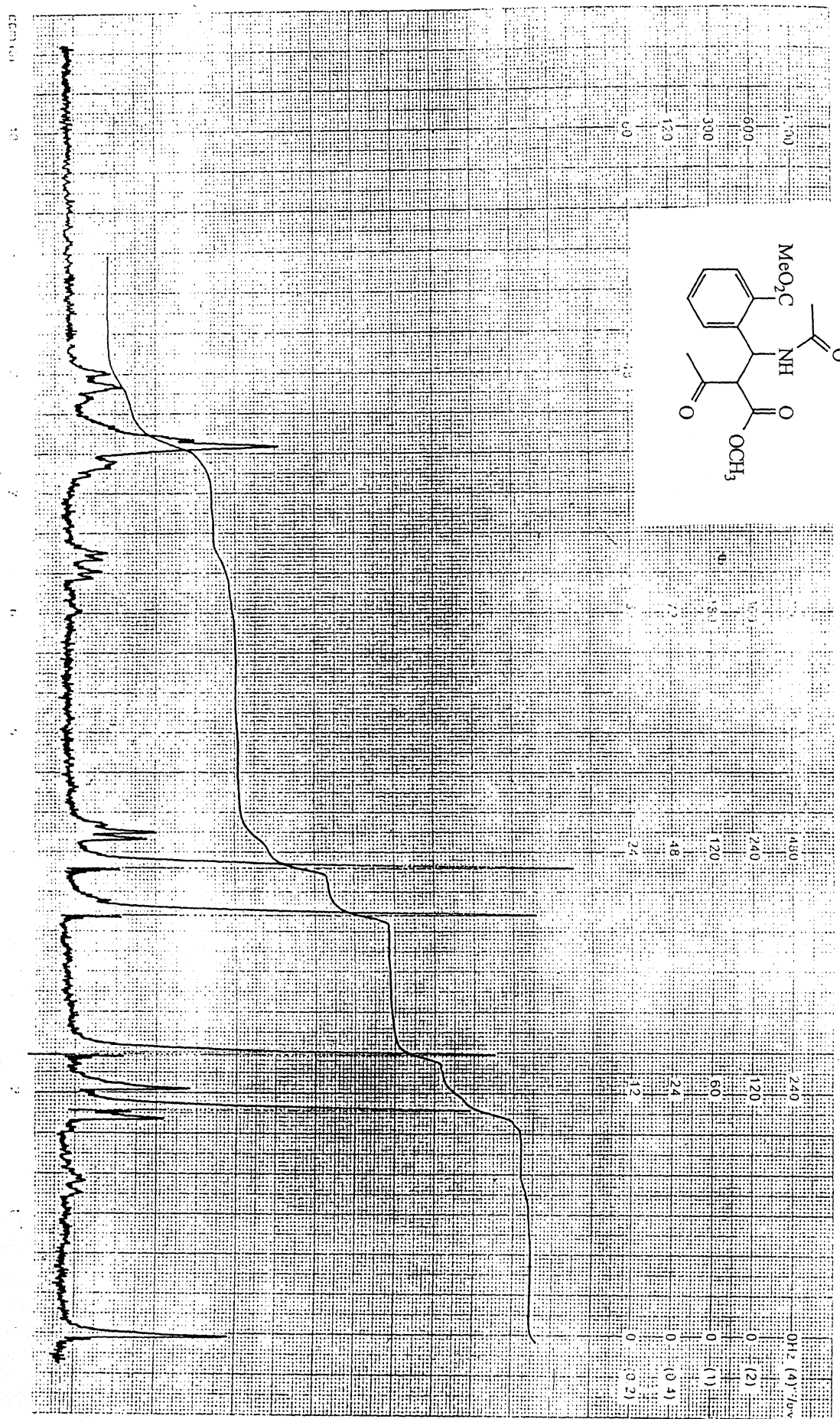




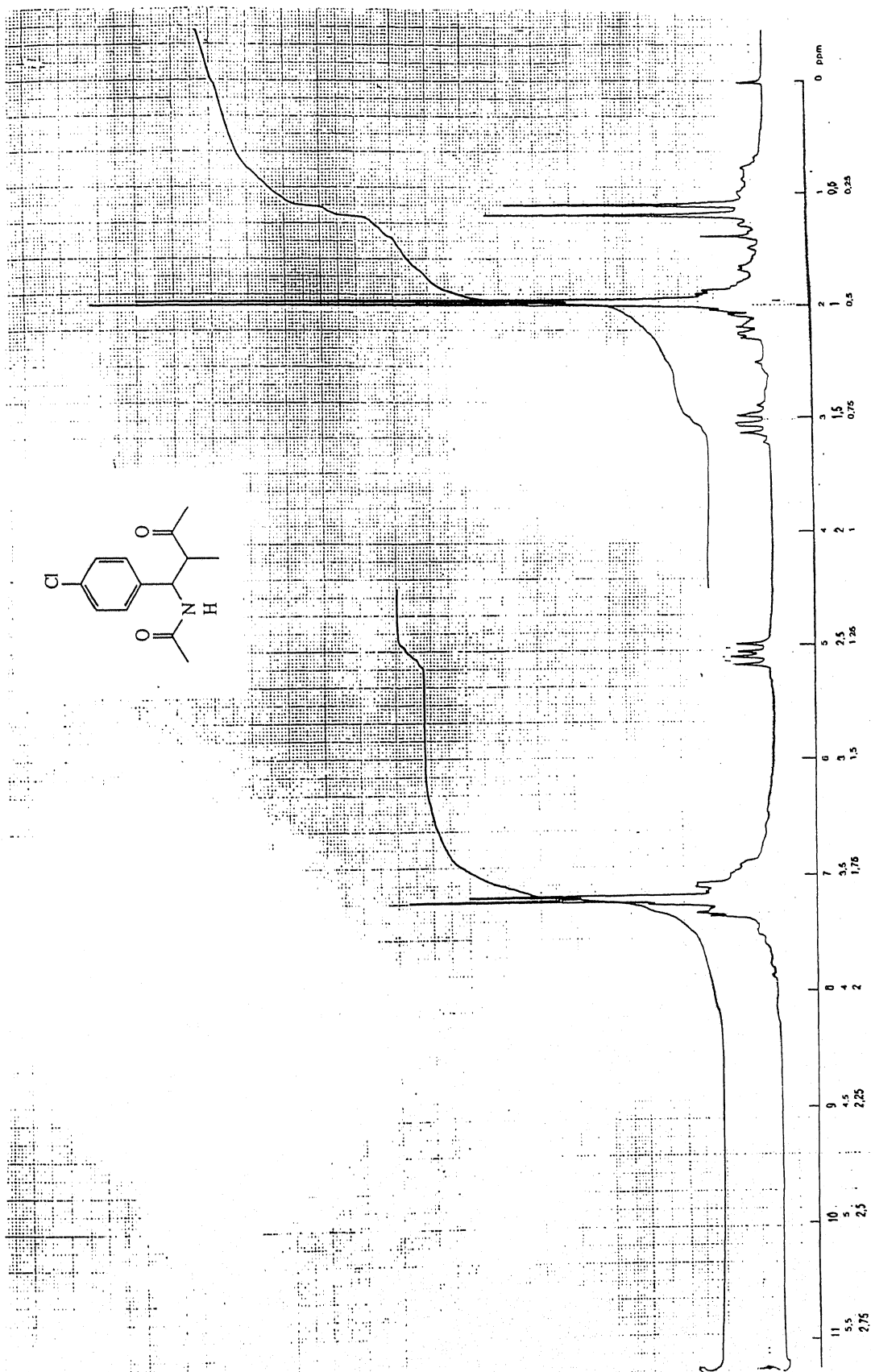
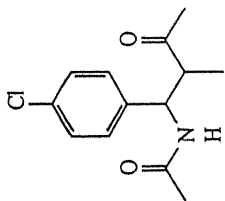


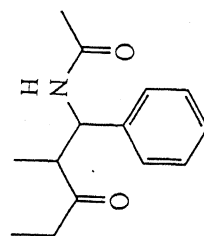
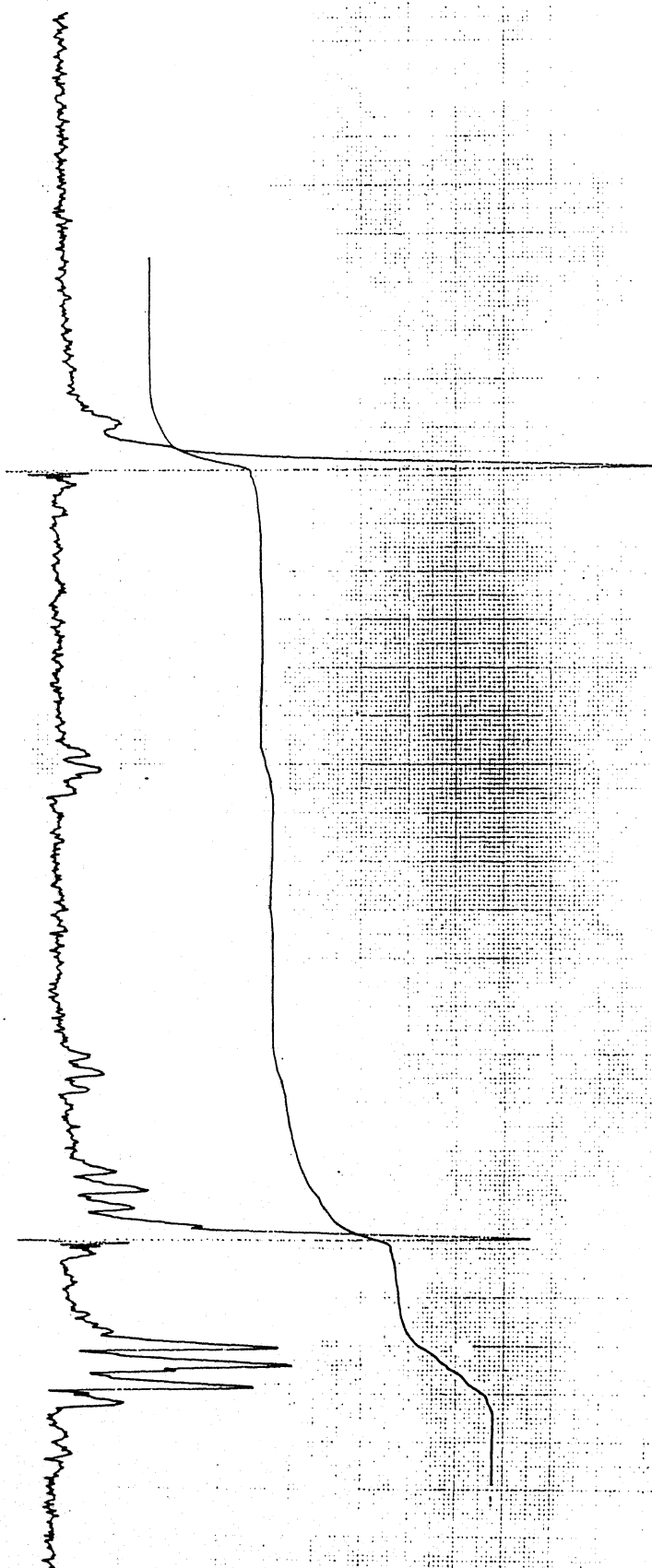


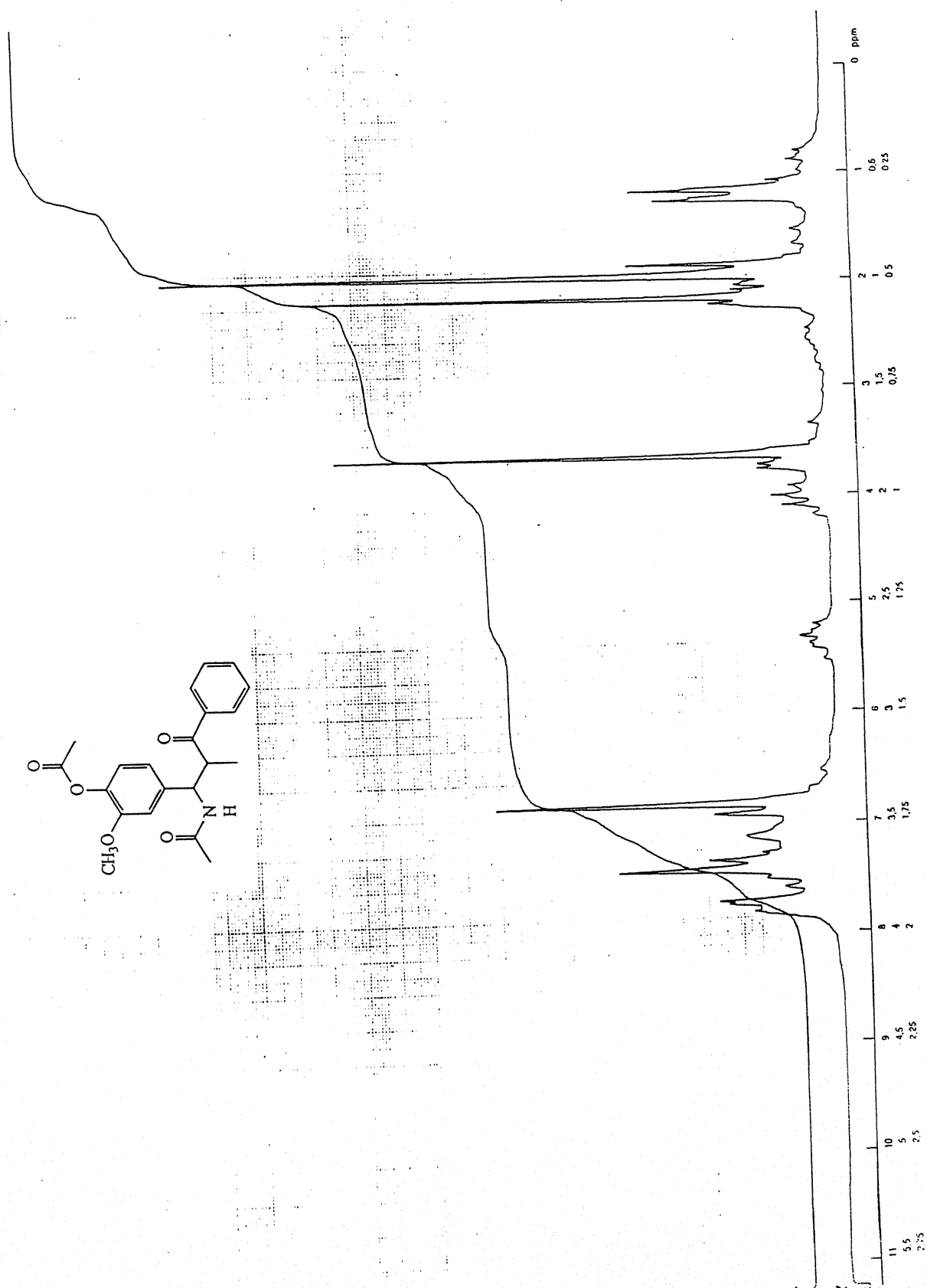
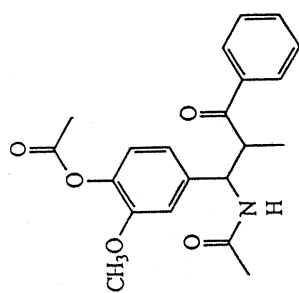


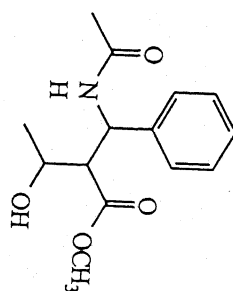
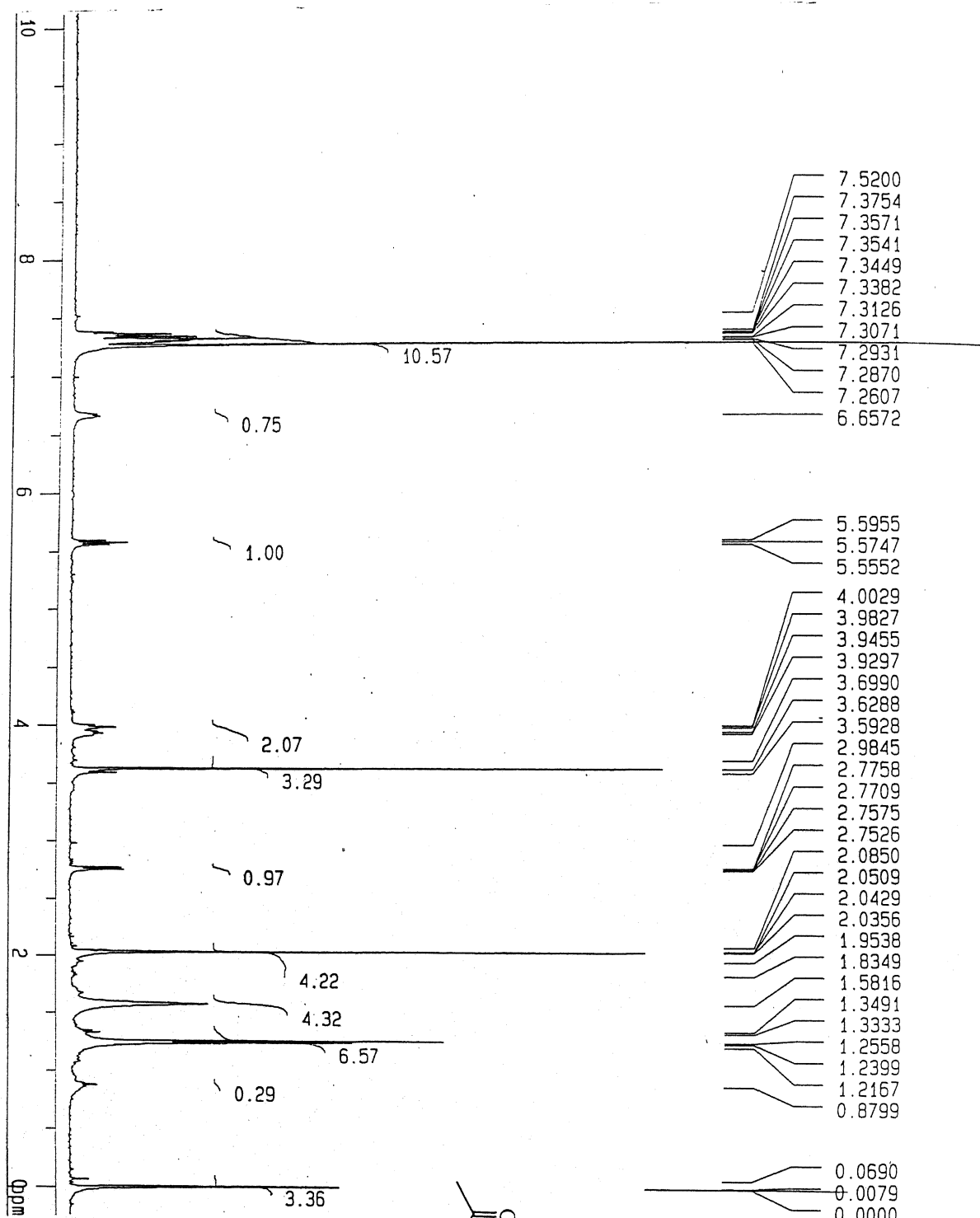


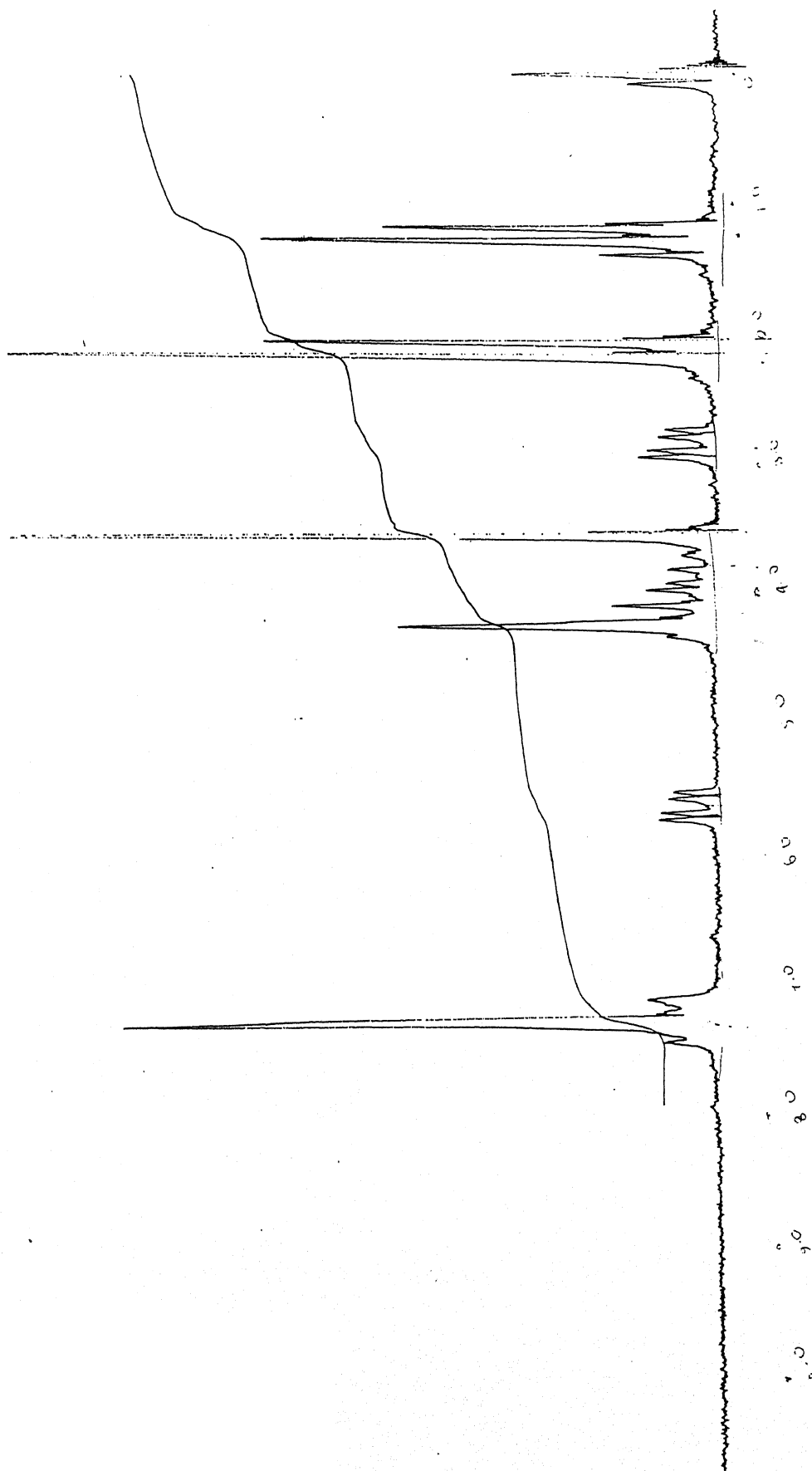
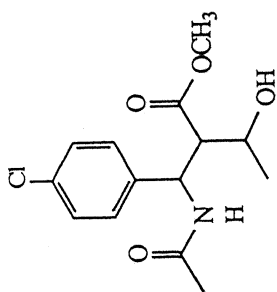
JNM-PMX 60 NMR SPECTROMETER

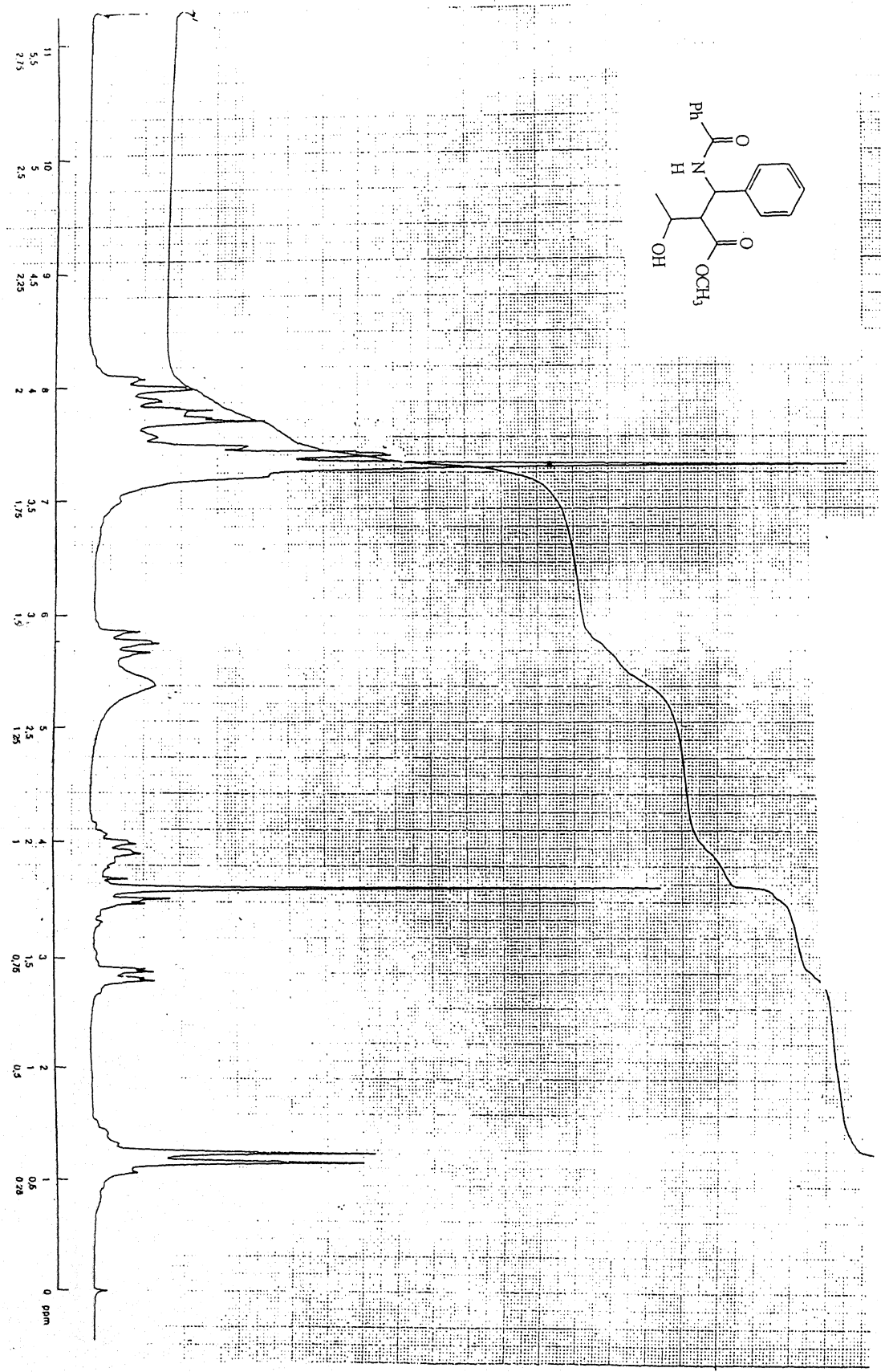
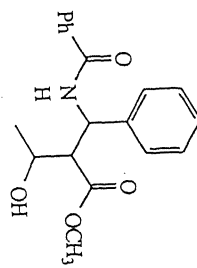


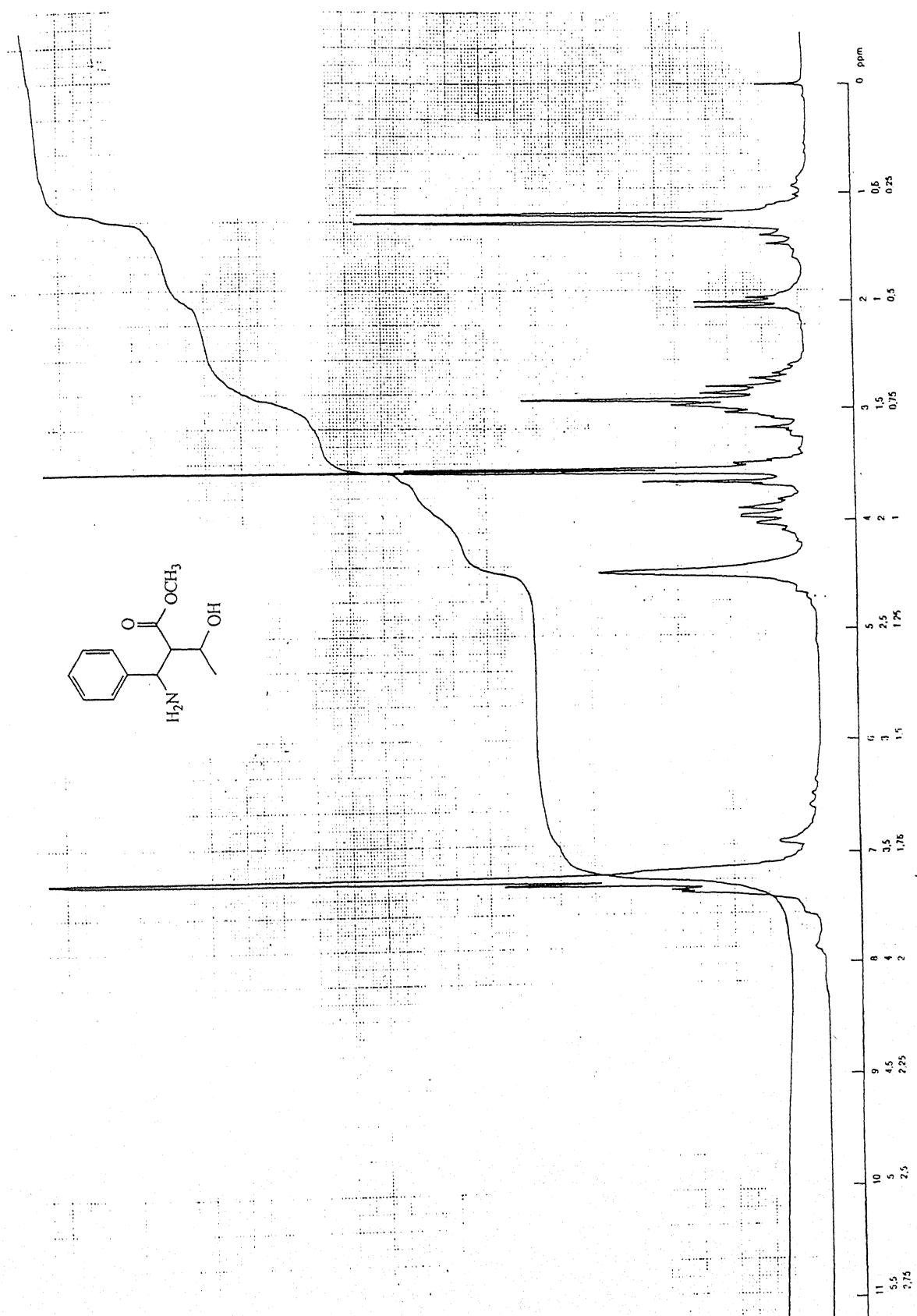
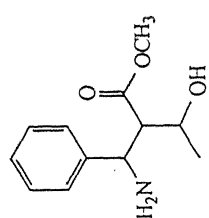


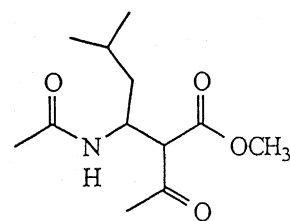
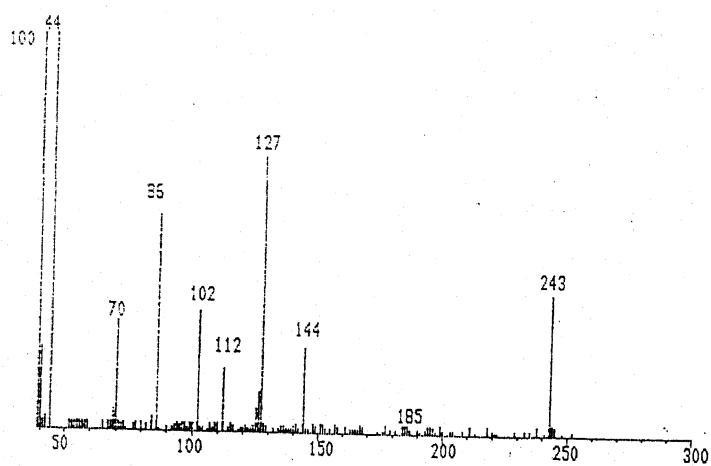
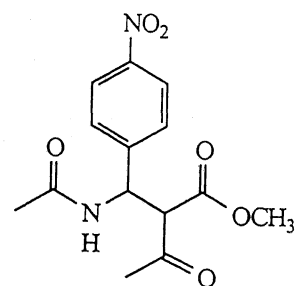
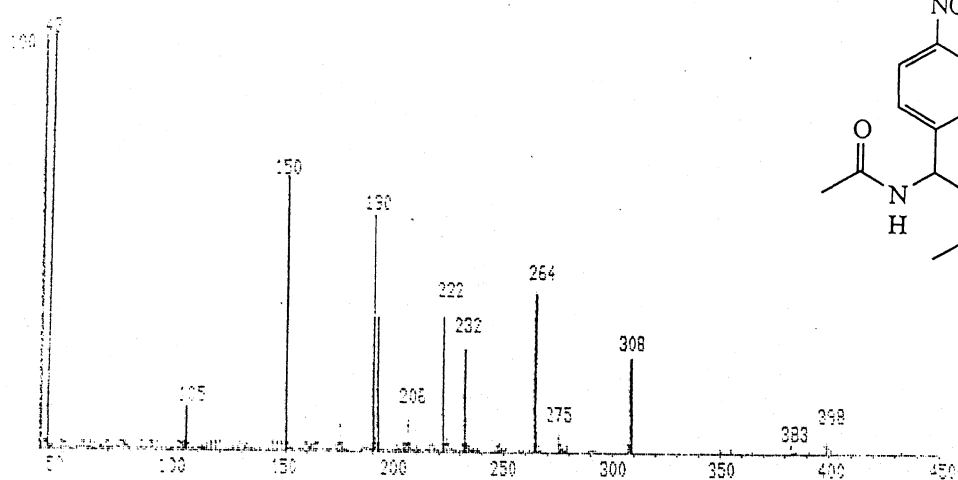
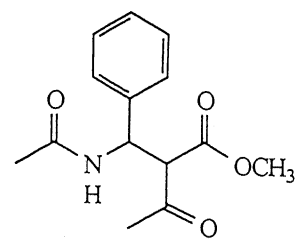
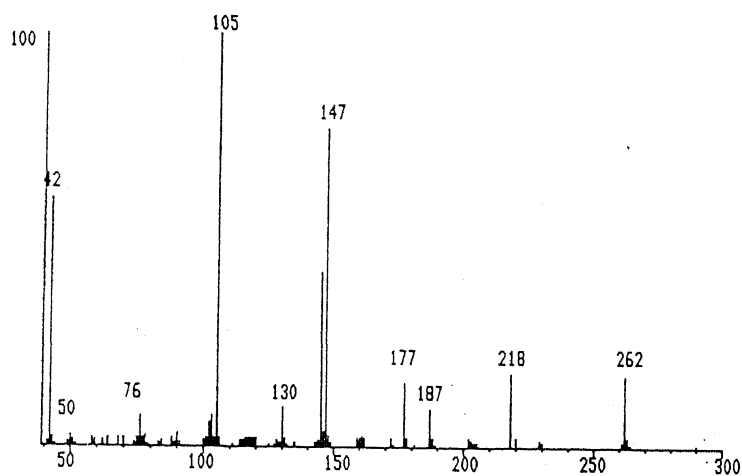


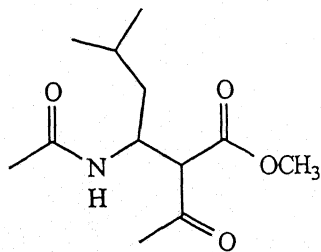
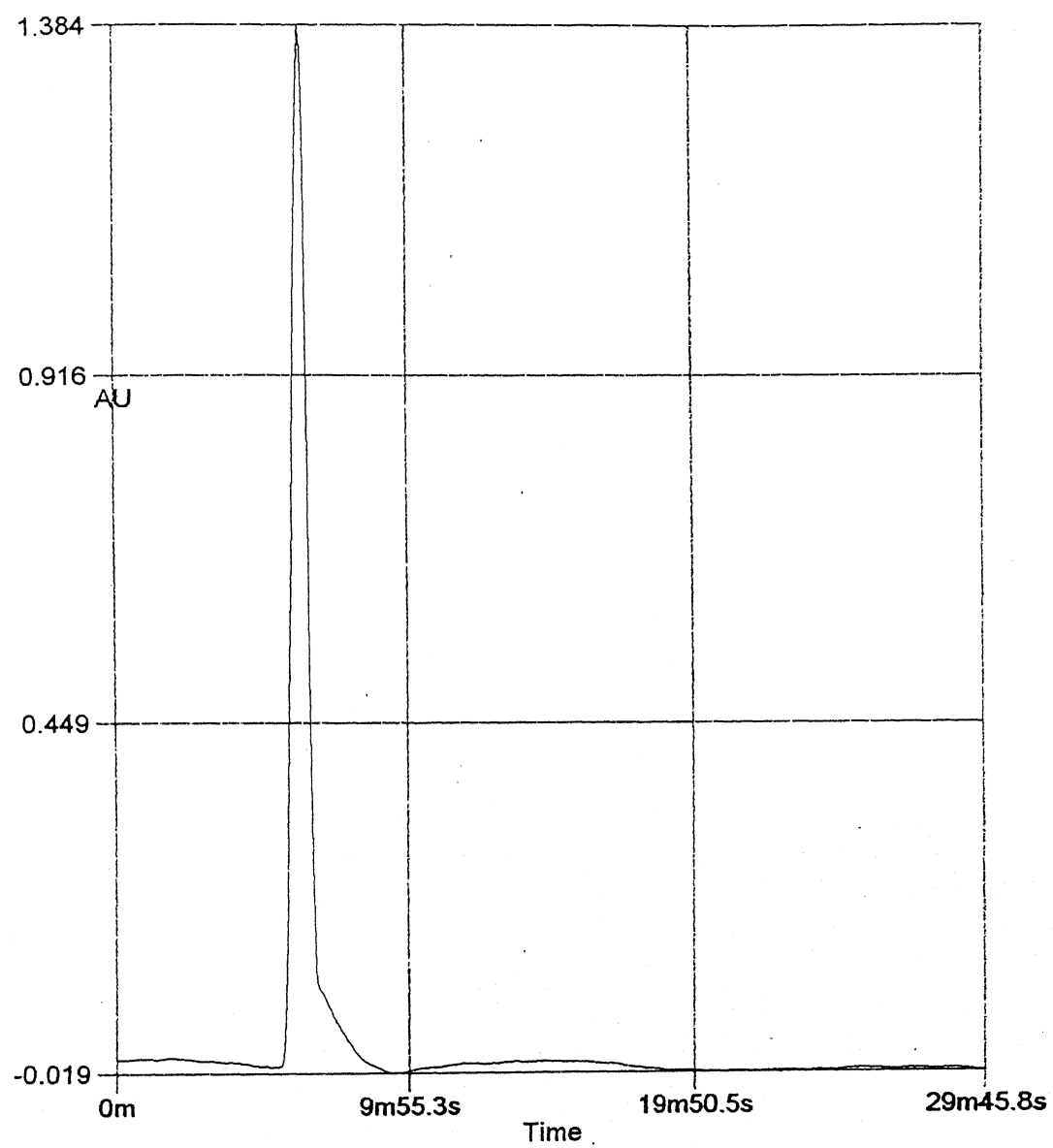




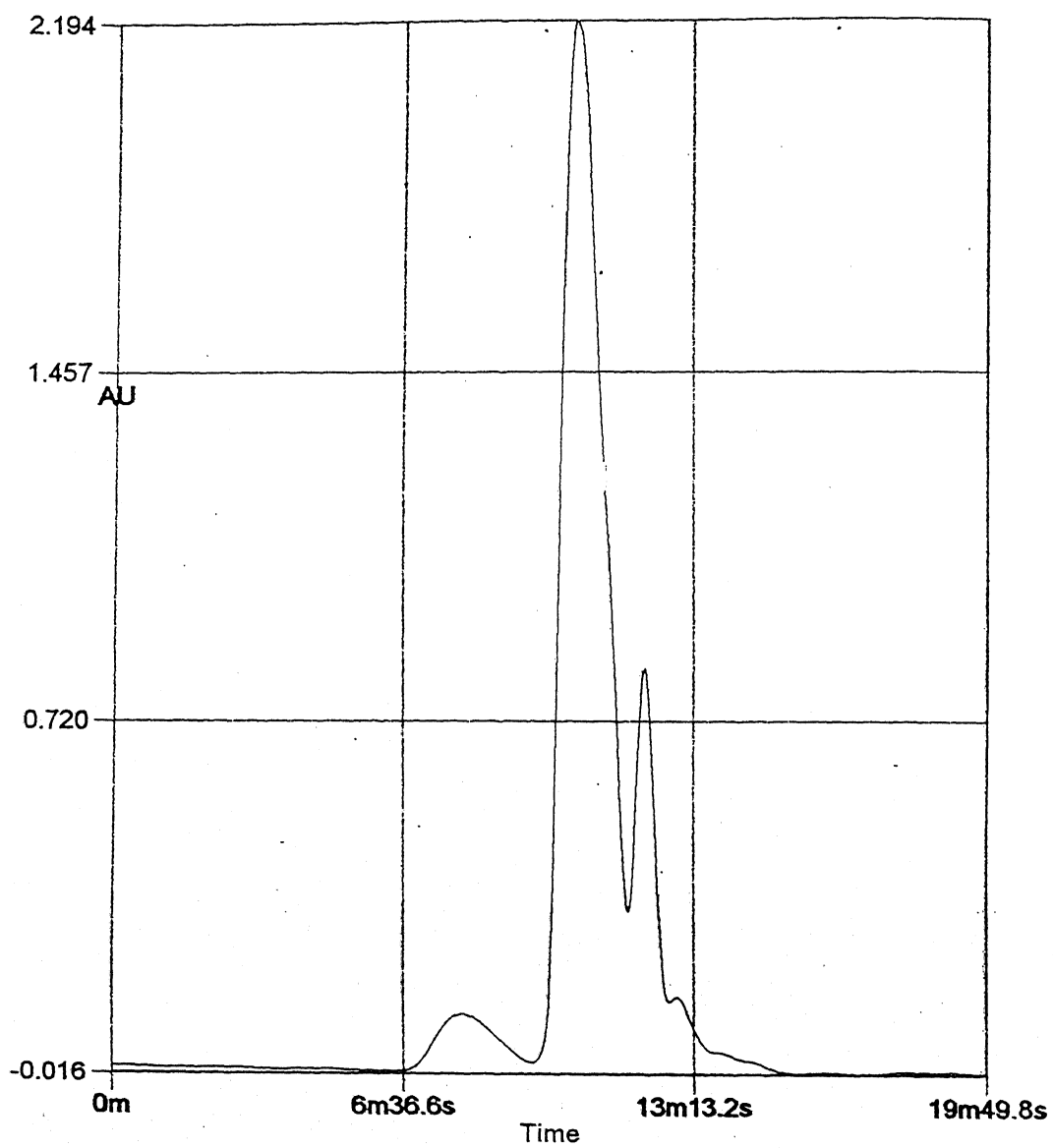




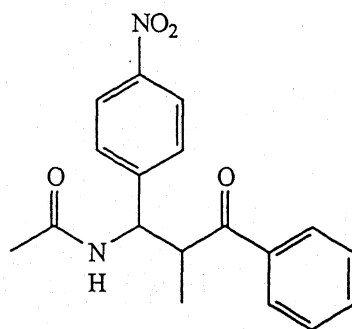


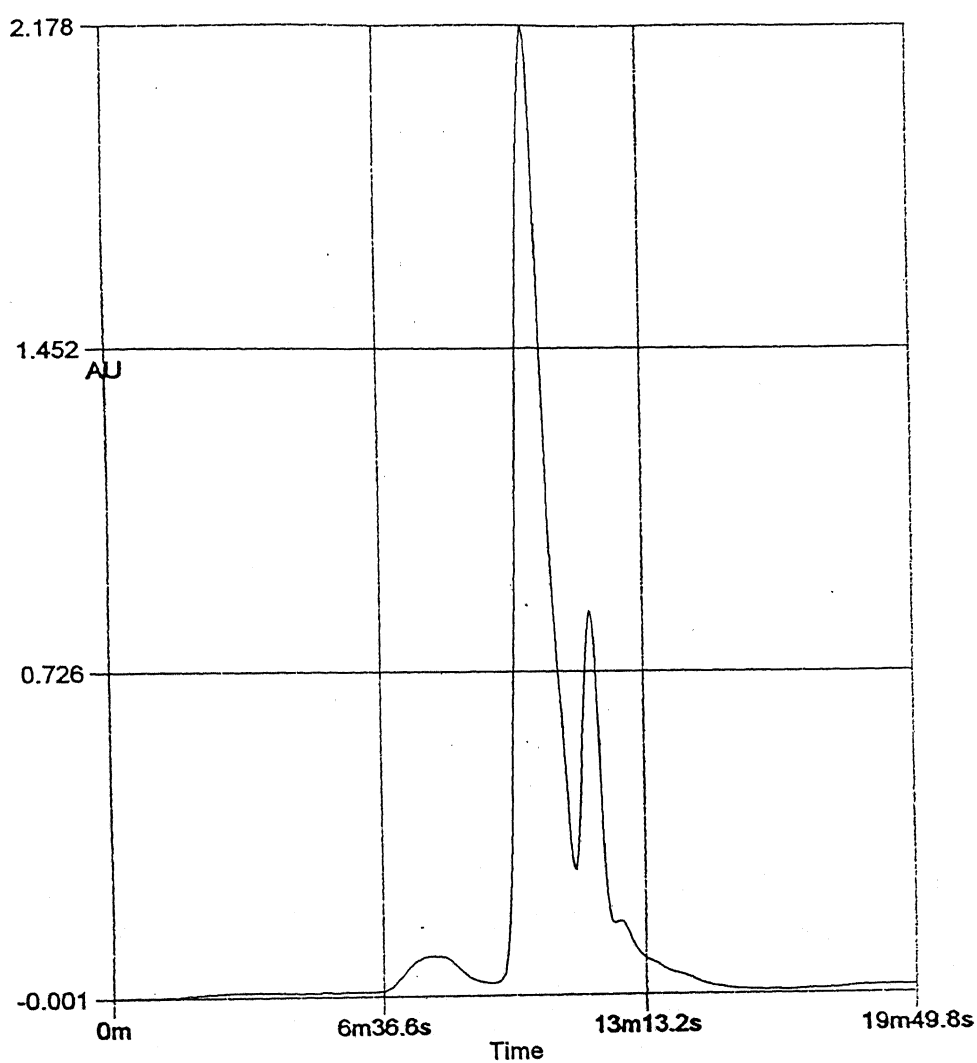


CHIRAL HPLC

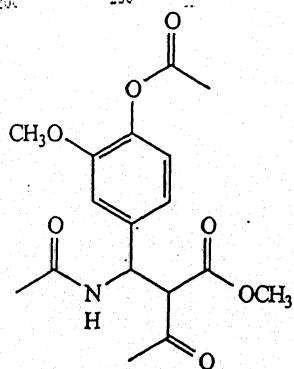
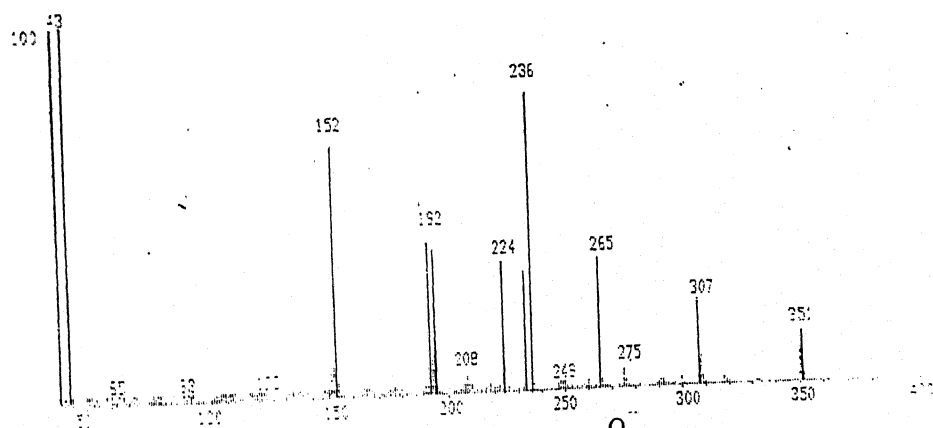


CHIRAL HPLC





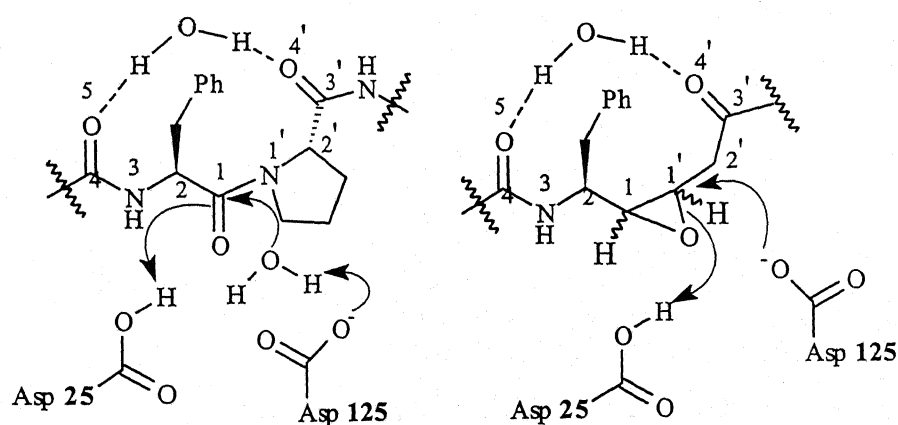
CHIRAL HPLC



**POLYANILINE SUPPORTED COBALT SALEN
CATALYSED SYNTHESIS OF β -PHENYLISOSERINE
DERIVATIVES CONTAINING HMC ISOSTERE: REGIO-
AND CHEMO-SELECTIVE OPENING OF EPOXIDES FOR
THE SYNTHESIS OF MACROCYCLIC PRECURSORS**

Introduction to Irreversible Inhibitors

A shadow has been cast on the promise of potent inhibitors for the HIV-PR now available by the finding that HIV becomes resistant to the inhibitors by evolving strains encoding protease variants that are resistant to the inhibitors.^{183,184} Most of the reversible inhibitors that exist today have very fast dissociation rates, which indicate that the inhibitory activity could be maintained only by enough concentration of the inhibitor at the drug target site.¹⁸⁵ In light of this development, one of the recent and widely appreciated approaches is the design of irreversible inhibitors of the enzyme. This is expected to be less susceptible to development of resistance by the protease, since irreversible binding of the ligand would lead to the "destruction" of secondary structure and hence the active site of the enzyme.^{186,187} Also, irreversible inhibitors would be more desirable in the aspect of the long time *in vivo* inhibitory efficacy which reversible inhibitors might not offer. For this, assistance from the reactive residues of the enzyme (protease) is essential. The most desirable amino acid residues for reaction with irreversible inhibitors, in HIV PR are its catalytic aspartate groups because mutations of these residues have been shown to suppress catalytic activity of the enzyme. On the other hand, irreversible inhibitors are required to comprise of a reactive functionality, or a latent version of such functionality as part of a ligand framework for suitable interactions with the enzyme, leading to irreversible binding with it.



Two dimensional model for the inactivation of HIV protease (right) based on the substrate hydrolysis mechanism (left)

Figure-1

The concerted general acid-general base catalysed mechanism for hydrolysis of the Phe-Pro peptide bond of substrates at the HIV PR active site has been discussed¹⁸⁸⁻¹⁹¹ earlier. Meek *et al* made use of this concerted mechanism for the design of irreversible

inhibitors¹⁹² of HIV PR. They reasoned that inactivation of the HIV PR is possible by the introduction of ligands (eg. epoxide) that can alkylate the Asp₂₅ and Asp_{25'} carboxyl groups at the active site (Figure-1). This is especially a potentially useful approach for circumventing the evolution of HIV strains that are resistant to protease inhibitors. Realisation of this in an inhibitor would require functional groups in the ligand, which are labile towards the direct nucleophilic addition of unprotonated aspartic residue coupled with the aid of the protonated one.¹⁸⁵ Meek *et al* demonstrated¹⁹² that 3-(4-nitro)phenoxy-1,2-epoxypropane (EPNP) irreversibly inactivated the HIV-1 PR with a maximal inactivation rate, K_{ina} of 0.004 min^{-1} and K_i of $11 \mu\text{M}$. Since then, several groups have come up with the design of many peptide analogues, incorporating the epoxy isostere as irreversible inhibitors of HIV-PR.

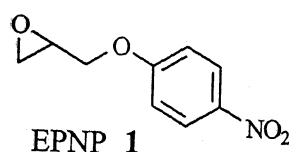


Figure-2

Haloperidol derivatives with α,β -unsaturated ketones in the flexible side chain have been shown to be irreversible inhibitors of HIV-PR.^{193, 194}

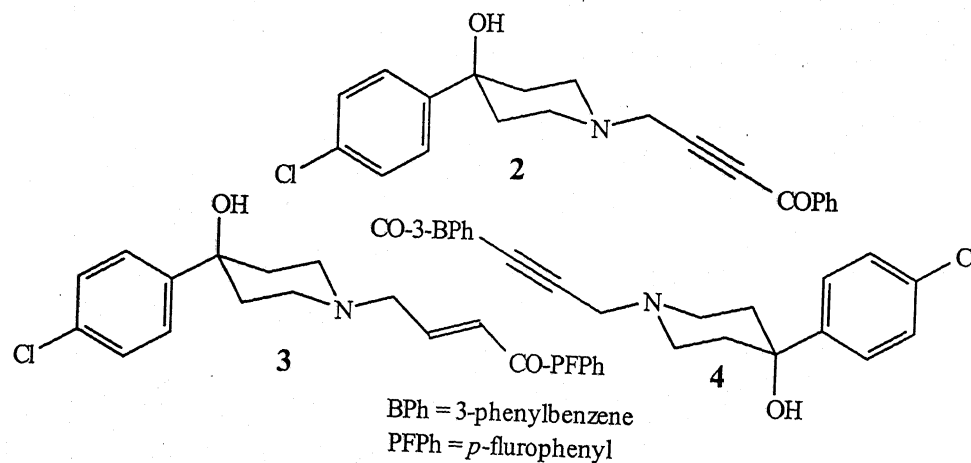
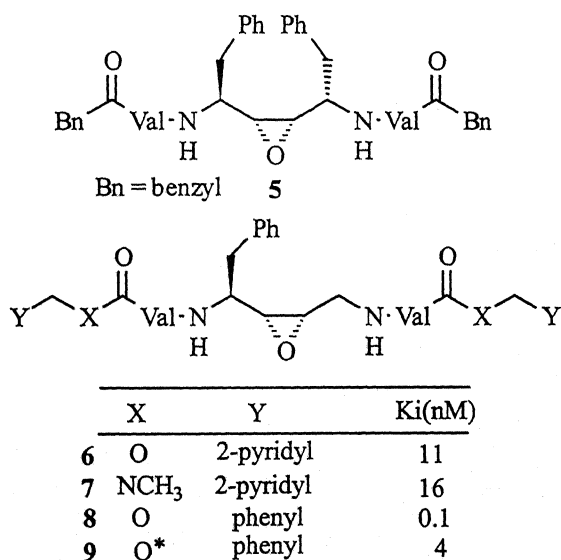
Structures of α, β -unsaturated ketone derivatives of haloperidol

Figure-3

It was learnt however, that inactivation of HIV PR by the α,β -unsaturated ketones involved (a) modification of both subunits of the protease dimer rather than only one subunit; (b) binding of more than one molecule of the inhibitor to the protease monomer;

and (c) modification of the cysteine residues (Cys-67 and Cys-95) and to a small extent the N-terminal amino group rather than the catalytic aspartate group.

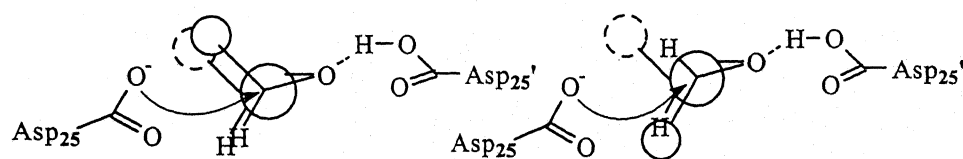
Considering the presence of C_2 -symmetry in HIV-PR, a C_2 -symmetric irreversible inhibitor was designed¹⁹⁵ and synthesized by mCPBA epoxidation of *cis* olefin. The epoxide served as the isostere for the Phe-Phe amide bond. Further studies showed that phenylalanine did not suit the P_1' site. Lead optimization using molecular modeling, inhibitory and binding studies of the symmetric inhibitor, led to the discovery of a potent HIV-PR inhibitor **8**, with high affinity and irreversibility ($IC_{50} = 30$ nM). **8** incorporates a *cis* epoxide as isostere at the Phe-Gly peptide bond.



Structure and potency of Irreversible *PSEUDO*- C_2 -Symmetric HIV-PR Inhibitors * P' site was cyclohexylalanine

Figure-4

Epoxides as Structure and Mechanism based inhibitors



Proposed Mechanism for Hydrogen-bonding-assisted alkylation of the catalytic Aspartate group by 1,2-disubstituted epoxides
Higher reactivity of *cis*- relative to *trans*- owing to steric effects

Figure 5

In a study of steric and energy factors¹⁸⁶ involved with the hydrogen-bonding-assisted alkylation of the catalytic aspartate groups by 1,2-disubstituted epoxides, *cis* epoxide has been proposed as a better mechanism based irreversible inhibitor of aspartyl proteases than the *trans* epoxide, due to its higher reactivity owing to steric factors. A model has

been presented for the alkylation mechanism (Figure-5), based on the results and finding of hydrolysis of epoxides by epoxide hydrolases.^{188, 191}

Based on this mechanism and the studies of haloperidol derivatives, potent structural analogues of haloperidol have been synthesized which showed inhibitory activity against HIV PR.

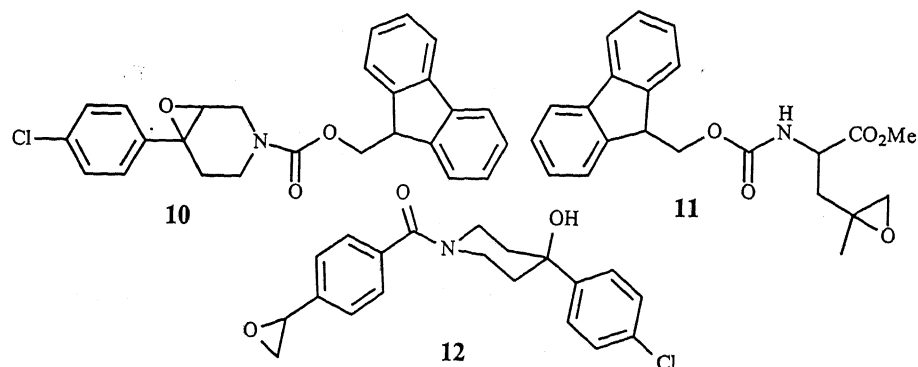
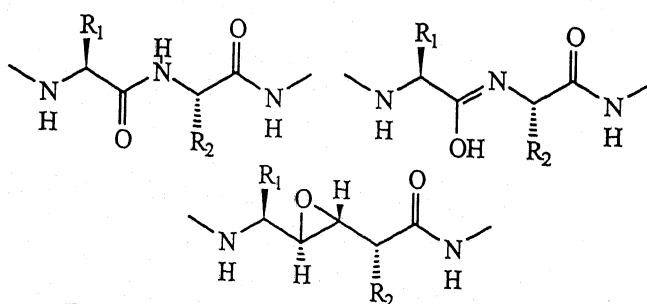


Figure 6

However, Taddei *et al* showed¹⁹⁶ (Figure-7) that *trans* epoxide is an efficient structural surrogate for a peptide bond. They propose the *trans* epoxide to be a structure-based transoid-hydroxy mimic that contributes to the transition state during the peptide cleavage by the protease.¹⁹⁷

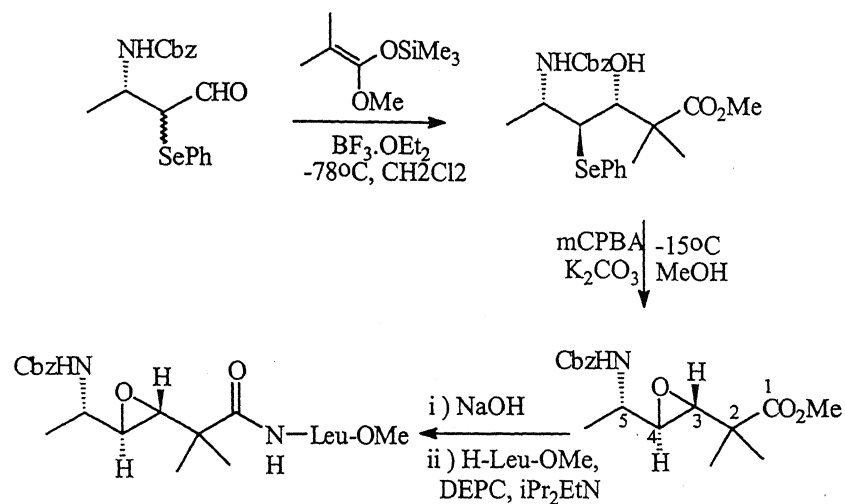
They have designed a "real" oxiranic peptidomimetic structure (13) where the three membered ring mimics the peptide bond inside the oligopeptidic frame; and propose that the *trans* oxirane could be more similar to the transoid hydroxy-mimic structure which contributes to the transition state during the peptide cleavage done by protease.¹⁹⁷



Trans epoxide as structure based peptidomimic

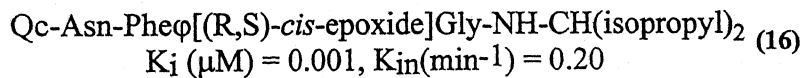
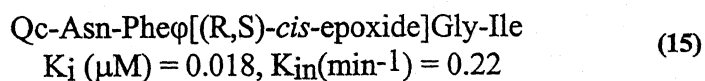
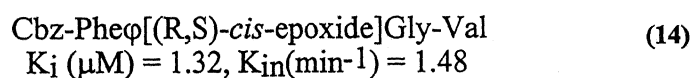
Figure-7

They have reported a general synthetic route (Scheme-1) for the synthesis of oligopeptides containing a *trans* oxirane ring in place of the peptide bond, prepared by oxidative conversion of β -hydroxyselenides obtained by Mukaiyama aldol type reaction of N-protected- β -selenyl aldehydes derived from naturally occurring amino acids.



Scheme-1

Kim *et al* reported the high binding and inactivation of HIV PR by tripeptides containing *cis* epoxide as transition state isostere¹⁸⁵ (Table-1).



Qc = 2-Quinolinecarbonyl

Enzyme inhibition constants of various oxirane peptidomimic

Table-1

Novel irreversible inhibitors of HIV PR, containing sulfonamide and sulfone as amide bond isosteres have been designed and synthesized, based on the report of the potent irreversible inhibitor (17) by Lee *et al*.¹⁸⁵

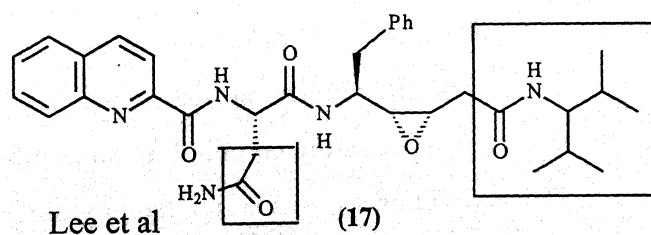


Figure-8

regio-selective intramolecular ring opening of epoxides²⁰⁰, it may be noted that epoxides are very versatile intermediates for organic synthesis.

Epoxidation of an alkene containing one or more chiral centers can furnish predominantly one of the two possible diastereomeric epoxides, depending on the face from which the reagent approaches the π -bond (Figure-11). If the two faces of the π -bond are unequally shielded, and if polar and

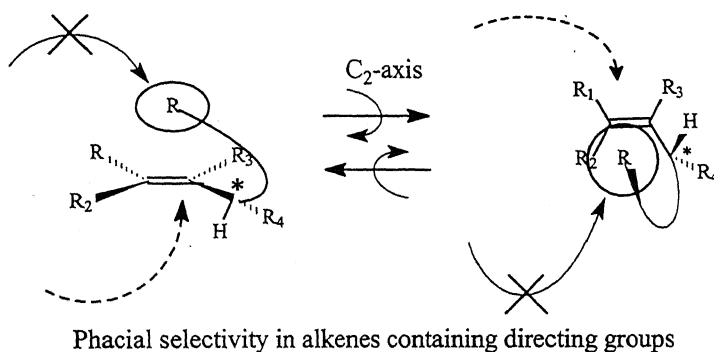
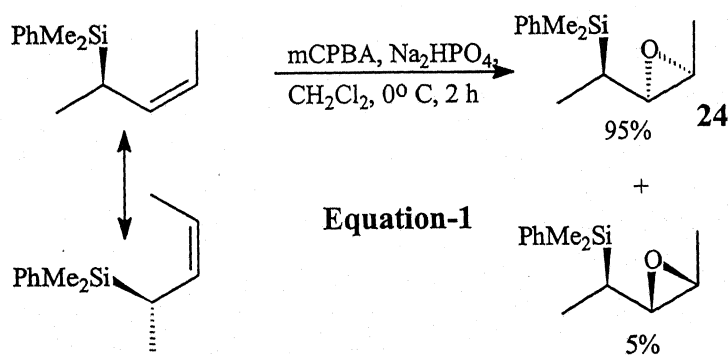


Figure-11

stereoelectronic factors are also involved, the two expected diastereomers will not be formed to the same extent, thus resulting in diastereoselectivity. An inspection of the molecular model may often reveal the face of the alkene that is more shielded, especially when the alkene has a rigid structure. Several factors, like the functional group, catalyst, chirality of the substrate, etc., control the stereoselectivity of epoxidations.

Substrate Directed Asymmetric Epoxidation

Epoxidation of chiral allylsilanes (eg. 23) are known to be stereospecific (for example, see Ref.201) (Equation-1).



The conformational preferences and stereoselective reactions of a number of macrocyclic systems have been studied. The stereochemical results have been explained on the basis of the model of local conformer control. Macrocycle containing a 1,5-diene system adopts a local conformation (Figure-12), which is free of torsional strain; epoxidation of

25 from the less hindered side furnishes the *syn*-epoxide **26**.^{202, 203} MCPBA epoxidation of tetra-methyllimonene **27** (unlike that of limonene) is regio- and stereo-selective²⁰⁴ (Equation-2) approach of the reagent from the β -face is blocked by pseudo axial

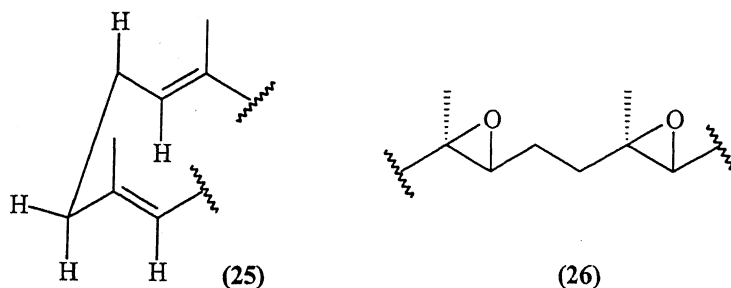
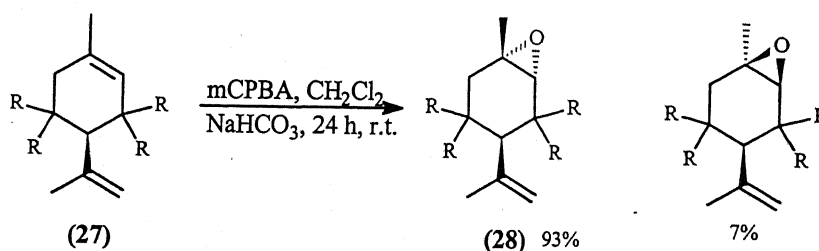
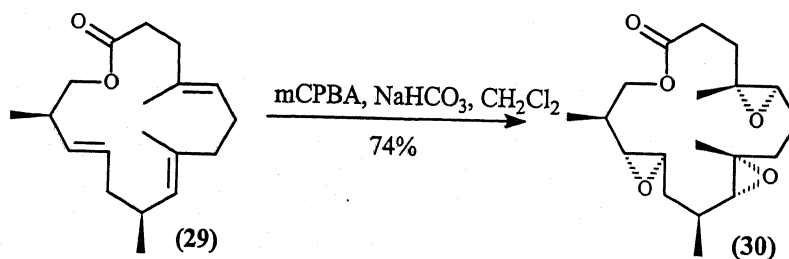


Figure-12

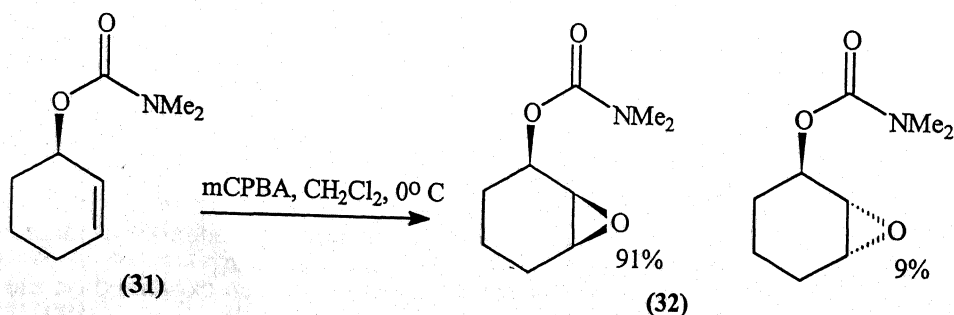
methyl at the C-3 and the axial methyl at C-5 a feature absent in limonene. Similarly, the mCPBA epoxidation of the unsaturated macrocyclic lactone **29** is stereoselective (Equation-3).



Equation-2

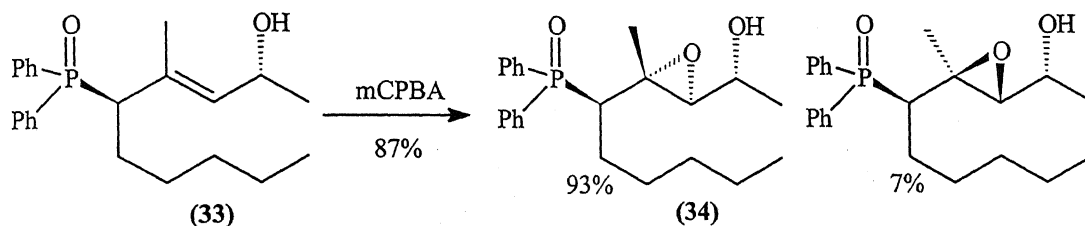


Equation-3

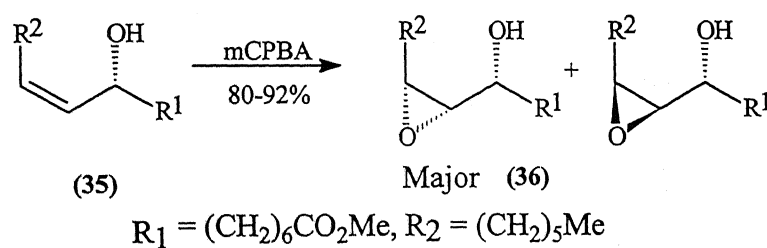


Equation-4

Alkene **31** underwent carbamate-directed stereoselective epoxidation²⁰⁵ (Equation-4). High stereoselectivity has been observed^{206,207} during epoxidation of the allylic alcohol **33** (Equation-5) having a tri-substituted double bond and the allylic alcohol **35** (Equation-6) having a *cis*-disubstituted double bond.

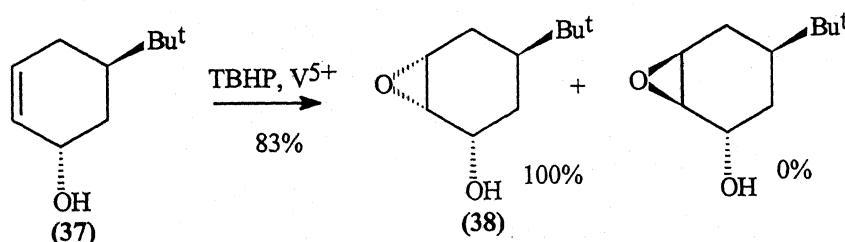


Equation-5



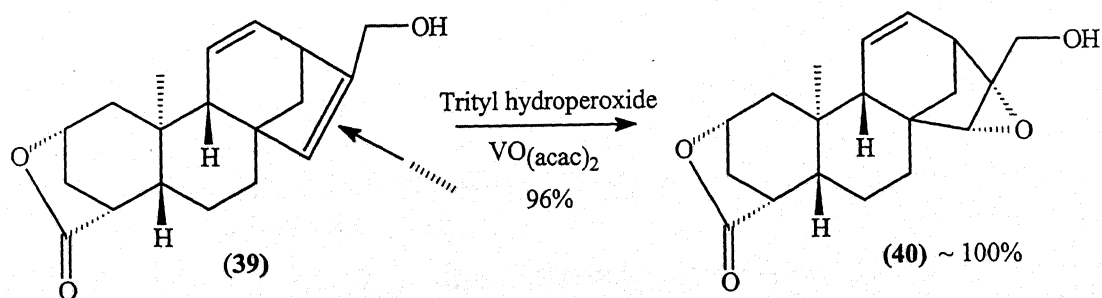
Equation-6

The epoxidation of the cyclic allylic alcohol **37** was stereoselective²⁰⁸ and took place from the face *cis* to hydroxy group (Equation 7).



Equation-7

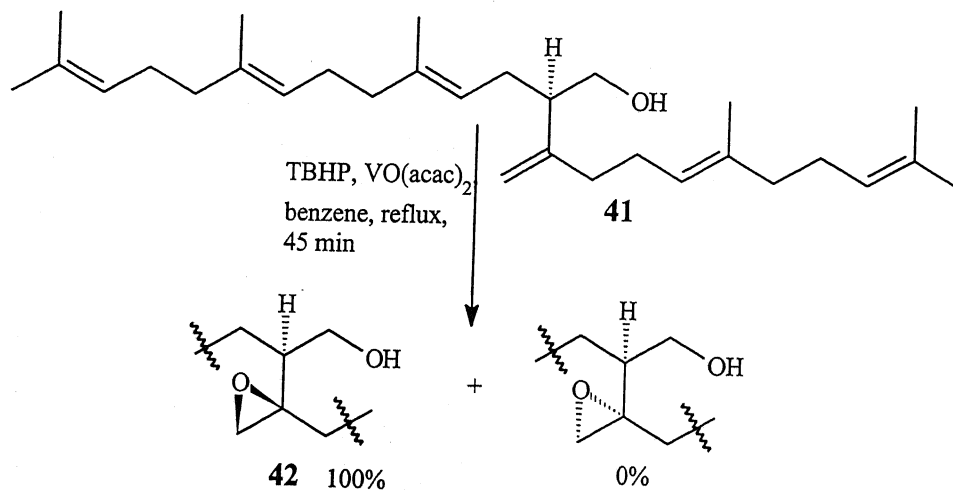
Similarly, the primary allylic alcohol **39** underwent hydroxy-assisted epoxidation, with



Equation-8

high stereoselectivity²⁰⁹ from the less hindered side to furnish the epoxide **40** in 96% yield, when it was reacted with tritylhydroperoxide/ $\text{VO}(\text{acac})_2$. (There was a decrease in stereoselectivity if TBHP was used instead of tritylhydroperoxide) (Equation-8).

The acyclic homoallylic alcohol **41** was epoxidised regio- and stereo-selectively²¹⁰ (Equation-9).



Equation-9

Catalyst Directed Asymmetric Epoxidation

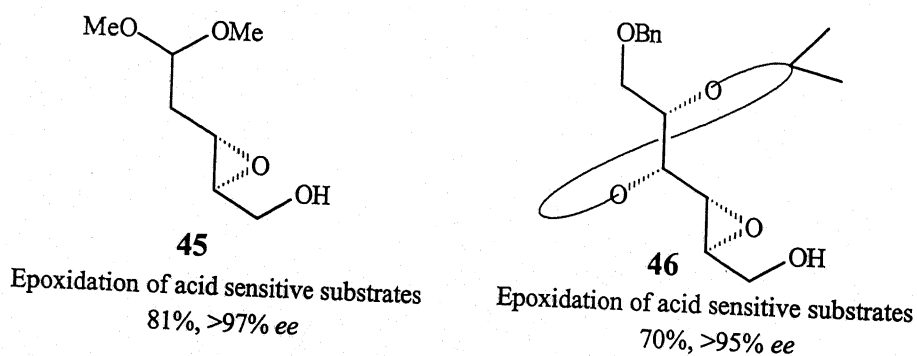
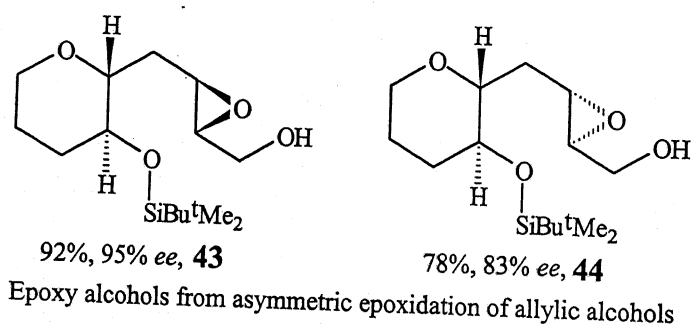
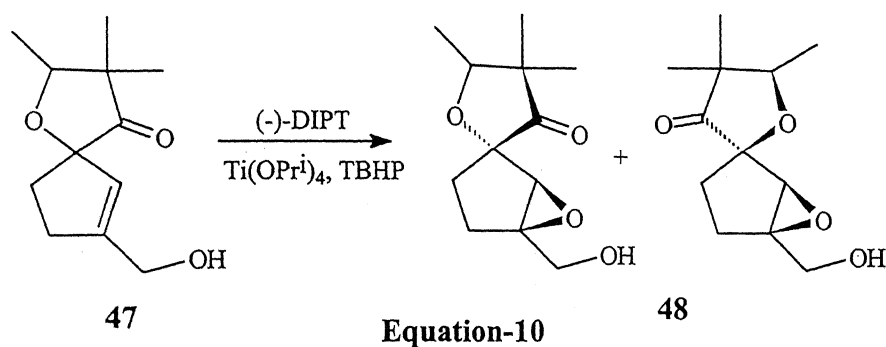


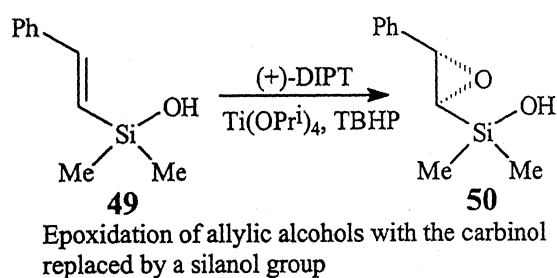
Figure-13

The Sharpless epoxidation methodology imparts chirality to prochiral allylic alcohols through a chiral complex formed between titanium isopropoxide, optically active

tartarate, t-butylhydroperoxide and the allylic alcohol. Compatibility of asymmetric epoxidation with sensitive functional groups like acetals, ketals and ethers has led to extensive use of allylic alcohols containing these groups in the synthesis of polyoxygenated natural products. Some examples have been presented.^{211, 212, 213}



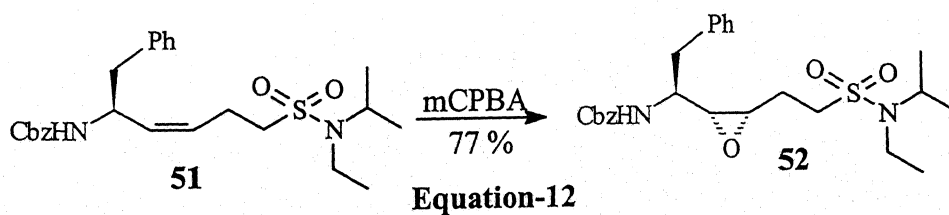
The asymmetric epoxidation of an allylic alcohol in which the carbinol has been replaced by a silanol has been described in high enantiomeric excess.²¹⁴



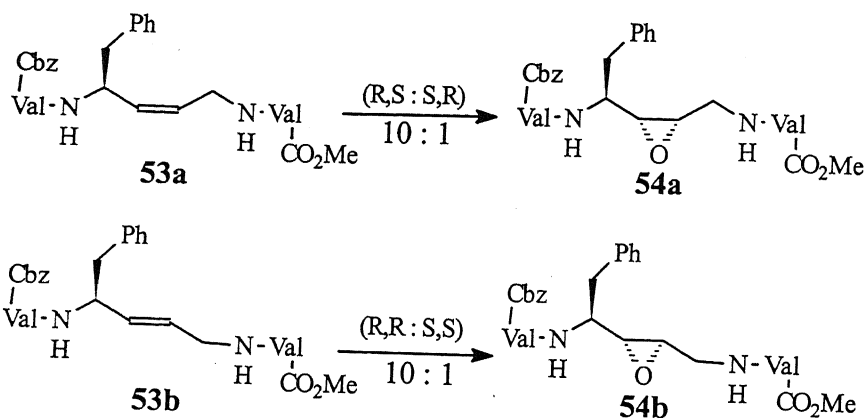
Enantioselectivity in epoxidation of peptides

Peptides are known to exist in semi-rigid secondary structures even in solution due to the partial double bond character of the amide C-N bond. The chirality of the aminoacid side chains has been used in several syntheses to lead to diastereoselective epoxidations.

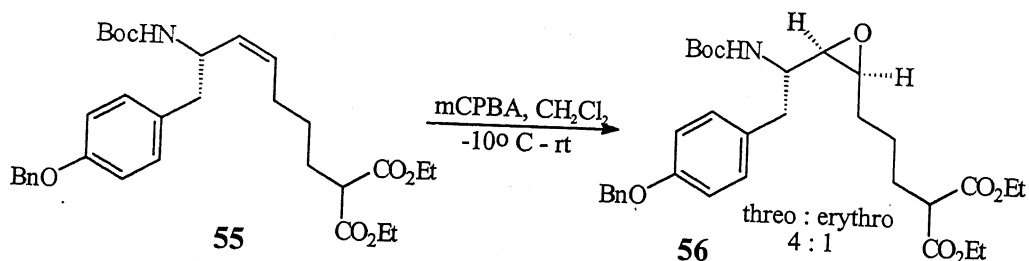
Epoxidation of the *cis* olefin **51** (Equation-12) with mCPBA gave the epoxide **52** as predominantly one diastereomer.^{215, 216, 217}



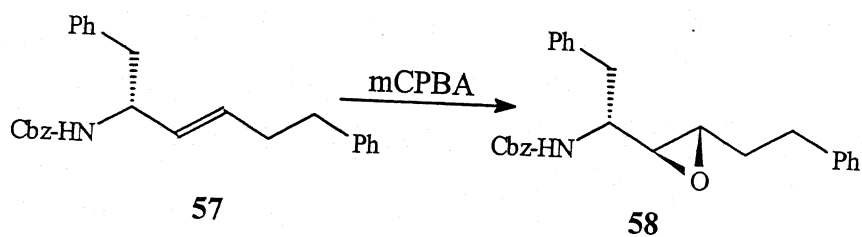
Epoxidation of the olefins **53a,b** was stereoselective to yield the expected diastereomers **54a,b** respectively²¹⁸ and was directed by the chiral phenylalanine moiety.

**Equation-13**

Predominantly threo-epoxy-malonate **56** was derived (Equation-14) from the *cis*- γ,δ -unsaturated precursor **55**, the selectivity being directed by the chiral tyrosyl side chain.²¹⁹

**Equation-14**

Stereochemistry of epoxidation can be solely directed by the stereochemistry of the amino acid side chain. Thus, replacing L-phenylalanine derivative with the unnatural D-phenylalanine derivative yielded the expected diastereoselective epoxide **58** in predominance.²²⁰

**Equation-15**

Present Study

The ability of HIV PR to synthesize resistant strains towards its existing reversible inhibitor ligands has kindled the idea and need for irreversible inhibitors. Oxiranes (epoxides) have been shown to possess just the right electronic requirements to serve our purpose in the design of irreversible inhibitors against aspartyl proteases. *Trans* epoxides serve as good structure based isosteres for the peptide bond. Hence incorporation of an epoxide moiety as part of a peptide framework has been the goal of several groups in recent past for the design and synthesis of potent irreversible inhibitors of aspartyl proteases, especially HIV PR. In this regard, we had earlier realized the potential of cinnamoyl group as suitable N-protecting group for amino acids as a source for generation of oxirane containing Phe-Xaa peptides via its subsequent epoxidation (Figure-1).

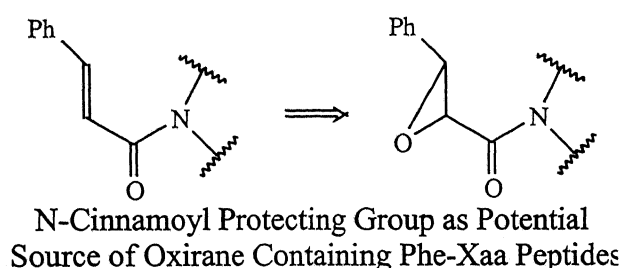


Figure-1

As can be seen from the forgone review,²²¹ most of the potent pseudopeptidic inhibitors of HIV PR are "tripeptides" containing three natural or unnatural aminoacids. A tripeptide sequence seems to be the optimum chain length for best inhibitory activity at the protease active site. It has been well recorded as preliminary studies from our group that cobalt catalyses the epoxidation of electron deficient α,β -unsaturated carbonyl and phenyl systems, in excellent yields.²²² Several reports, for the synthesis of "bestatin" analogues and "pyrrolidine-containing" dipeptide isosteres, have been reported by us²²² via the epoxidation pathway in the presence of polyaniline supported cobalt catalyst. We present below, our efforts and success in introducing *trans* oxiranes as part of tripeptide systems through polymer supported cobalt (II) salen catalysed epoxidation of N-cinnamoyl dipeptides. Extending the epoxidation protocol of polyaniline supported cobalt catalysed (especially by polyaniline supported cobalt salen - PASCOS) epoxidation of N-cinnamoyl-protected mono-peptides, to the N-cinnamoyl-protected dipeptides would result in generation of small-molecule-library of tripeptides,

incorporating the oxirane as part of the tripeptide. That the HIV PR is uniquely specific, unlike other mammalian endopeptidases, to the cleavage of Phe-Pro peptide bond **A**, is well recorded. Hence, the epoxidation of N-cinnamoyl-dipeptides containing the N-cinnamoyl-proline moiety would lead to the synthesis of small molecule library of tripeptides **B**, that contain an oxirane as isostere for irreversible binding with the target enzyme, adjacent to the scissile Phe-Pro amide bond (Figure-2). In general, the epoxidation of N-cinnamoyl-dipeptides containing the N-cinnamoyl-Xaa-Pro-moiety would lead to a small molecule library of tripeptides **C**, that contain an oxirane as isostere adjacent to the scissile Phe-Xaa amide bond (Figure-2), where Xaa is the amino acid of our choice.

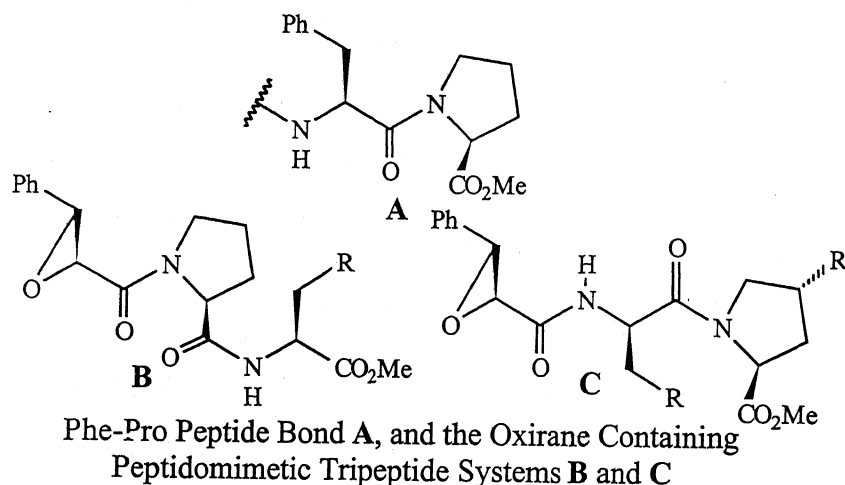
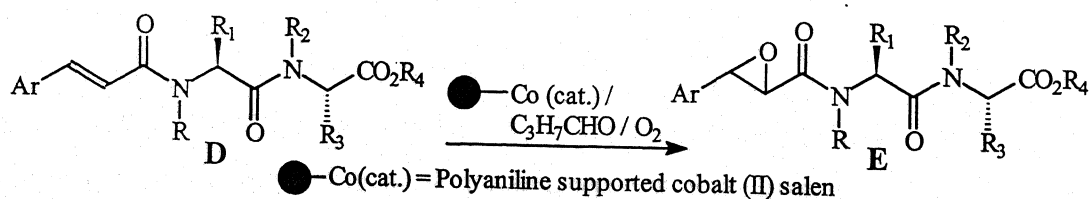


Figure-2

PASCOS catalysed epoxidation of N-cinnamoyl dipeptides-

Hence, using the epoxidation protocol in the presence of PASCOS and 2-methylpropanal in oxygen atmosphere, we have generated a library of small molecule tripeptides **E**, incorporating the trans oxirane as structure based peptide isostere, from the corresponding N-cinnamoyl-dipeptides **D** (Scheme-1).



General Methodology for the Synthesis of Small Molecule Tripeptides **E**
Containing Trans Oxirane, From the N-cinnamoyl-dipeptide Systems **D**

Scheme-1

The focus of our work as mentioned earlier was to mimic the Phe-Pro scissile amide bond, uniquely cleaved by HIV PR; and in general the Phe-Xaa peptide linkage, for recognition at the protease active site. A cursory look into the primary sequence study of different cleavage sites showed that the most common aminoacids in the sequence, apart from Phe and Pro were Leu, Ile, Val and Asp. Since Proline is an essential residue, we set about the synthesis of these N-cinnamoyl-dipeptide libraries, with proline as the $i+1^{\text{th}}$ amino acid residue (called the CPX peptide systems, **1**) and with proline as the $i+2^{\text{th}}$ amino acid residue (called the CXP peptide systems, **2**) from the cinnamoyl group (i^{th} residue), where X is the amino acid residue of choice (Figure-3).

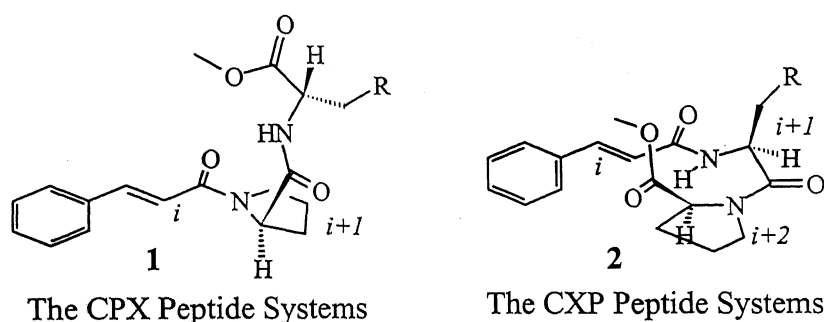
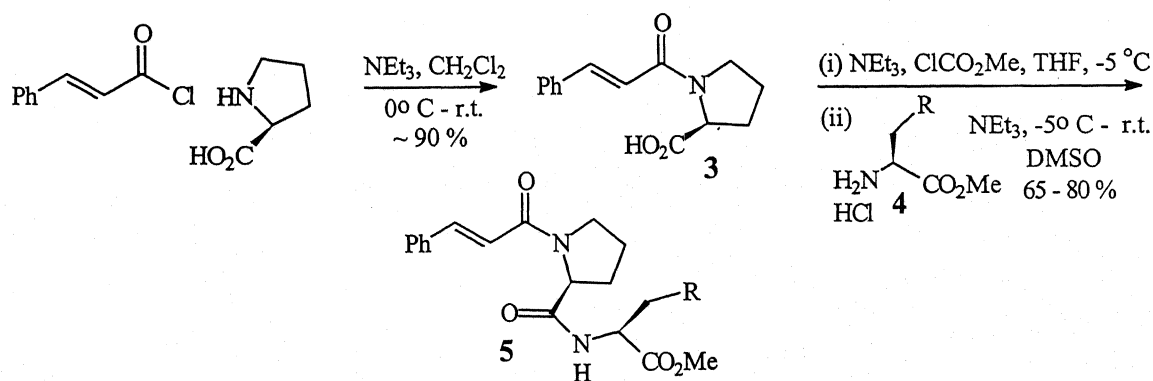


Figure-3

All these systems were synthesized via the mixed anhydride coupling procedure for the synthesis of peptides.

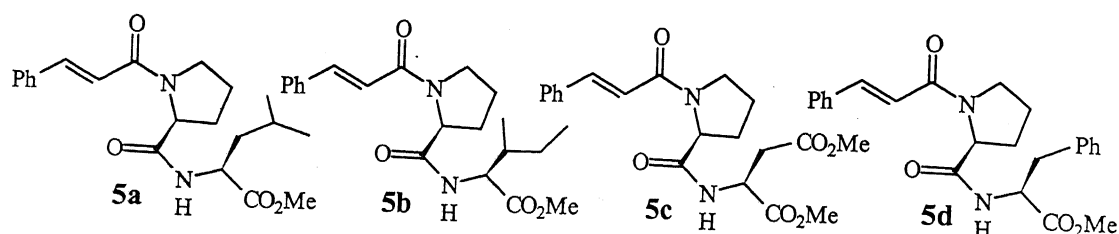
Proline as the $i+1^{\text{th}}$ Aminoacid Residue from Cinnamoyl group: The CPX Peptide Systems-



Scheme-2

All the CPX peptide substrates were synthesized by the mixed anhydride coupling procedure, with methyl chloroformate as the C-terminal activating group from N-cinnamoyl-L-proline. L-Proline was N-cinnamoylated with stoichiometric amount of

cinnamoylchloride and triethylamine in CH_2Cl_2 / CH_3CN , at 0°C . Purification by acidification and subsequent drying of the resulting precipitate yielded N-cinnamoyl-L-proline in excellent yields. N-cinnamoyl-L-proline **3** was subjected to mixed anhydride coupling with appropriate methyl-L-amino ester hydrochlorides **4** in THF, in the presence of triethylamine at -5 to -10°C to yield the corresponding methyl-N-cinnamoyl-(L)-proline-(L)-amino esters **5** in good yields after column chromatography (Scheme-2).

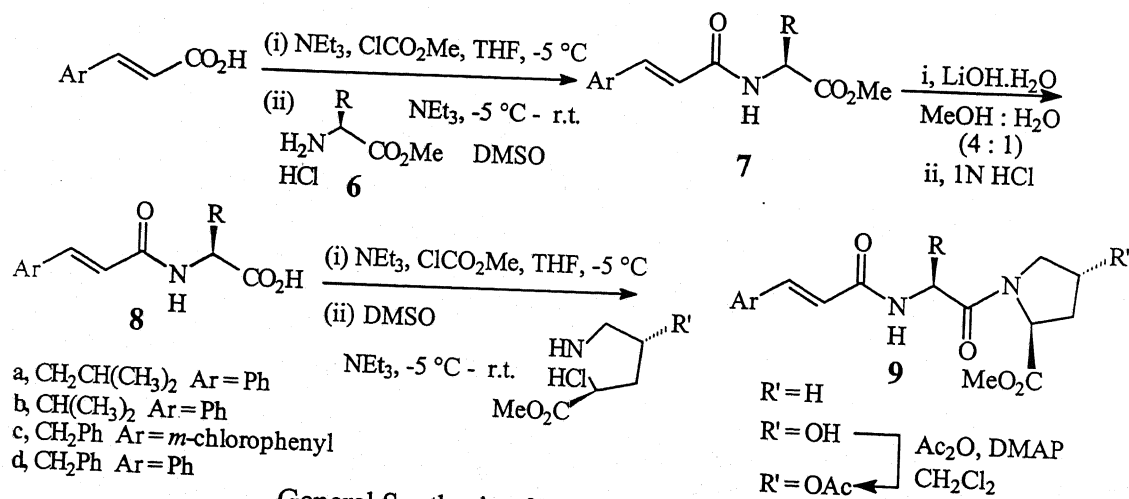


The CPX Peptide Systems

Figure-4

N-cinnamoyl-L-proline was coupled with methyl -L-leucinate, -isoleucinate, -aspartate and -phenylalinate hydrochloride (**4a-d**) using the mixed anhydride coupling procedure, to get to the CPX peptides **5a-d** (Figure- 4) respectively.

Proline as the $i+2^{\text{th}}$ Aminoacid Residue from Cinnamoyl Group: The CXP Peptide Systems-



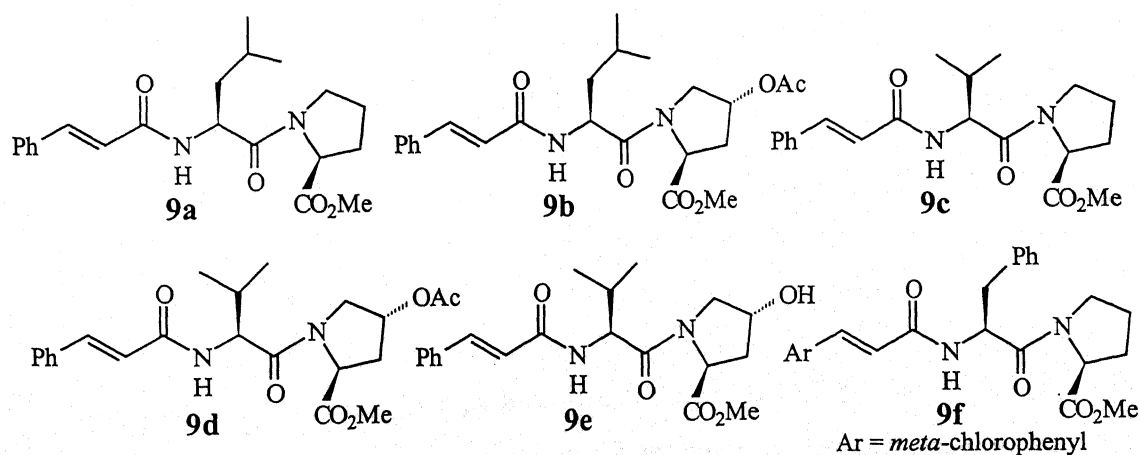
General Synthesis of The CXP Peptide Systems

Scheme-3

Methyl-N-cinnamoyl-L-Xaa-L-Prolinates were synthesized from N-cinnamoyl-L-Xaa acids by the mixed anhydride coupling procedure with appropriate methyl-L-prolinate hydrochloride of choice, using methylchloroformate as the C-terminal activating group.

In general, the CXP peptide systems were synthesized as shown in Scheme-3. Typically, cinnamic acid (or *meta*-chlorocinnamic acid) was converted to the corresponding activated mixed anhydride in THF, with methylchloroformate and triethylamine at -5 °C. Addition of the methyl-aminoester hydrochloride **6** of choice - dissolved in DMSO - followed by triethylamine, yielded the N-cinnamoyl aminoesters **7** in good yields after column chromatography. Base hydrolysis of the N-protected aminoesters with lithiumhydroxide and its subsequent acidification with 1N HCl gave the corresponding carboxylicacids **8** in near quantitative yields, which were coupled with methyl-L-prolinate hydrochloride or methyl-(L)-hydroxy prolinate hydrochloride as mentioned above *via* mixed anhydride coupling procedure with methylchloroformate as the C-terminal activating group, to yield the corresponding N-cinnamoyl-dipeptides **9** after column chromatography (Scheme-3). The acetylated hydroxyproline derivatives were synthesized by acetylation of the hydroxyproline derived N-cinnamoyl-dipeptides in dichloromethane with acetic anhydride in the presence of DMAP and triethylamine in appreciable yields.

Thus, the CXP systems (Figure-5) **9a-f** namely methyl-N-cinnamoyl-L-leucine-L-prolinate, methyl- N-cinnamoyl- L-leucine-(4-*trans*-oxyacetyl)-L-prolinate, methyl-N-cinnamoyl-L-valine-L-prolinate, methyl-N-cinnamoyl-L-valine-(4-*trans*-oxyacetyl)-L-prolinate, methyl-N-cinnamoyl-L-valine-(4-*trans*-hydroxy)-L-prolinate and methyl-N-(*meta*-chlorocinnamoyl)-L-phenylalanine-L-prolinate, were synthesized by the above procedure in good yields. Synthesis of **9f** was achieved by using *meta*-chlorocinnamic acid instead of cinnamic acid for N-protection of methyl-L-phenylalaninate.



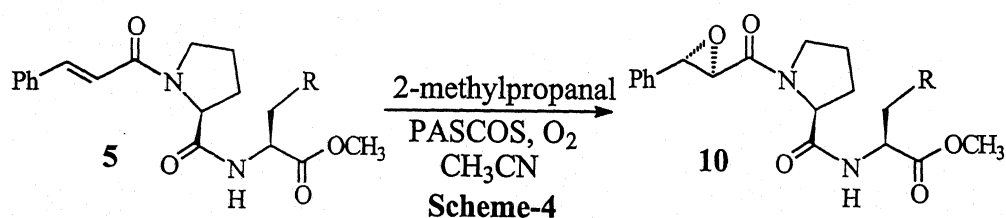
The CXP Peptide Systems

Figure-5

In general, the yields of CPX systems were observed to be better than the yields of CXP systems. Methylchloroformate may not be best of choices for the activation of carboxyl group during coupling. The reaction suffers in general from the formation of small to reasonable proportions (~5-15%) of the corresponding methyl esters from carboxylic acids, apart from the coupled peptide, which need to be column chromatographed. However, we justified the choice of methylchloroformate for activating the carboxylic acid during coupling due to its easy availability and cost, which is much lesser than that of some of the other related coupling reagents. In general, it was observed that yields of formation of peptides could be bettered and kept high on maintaining the reaction temperatures strictly below -10 to -5 °C. Using other coupling reagents, like isobutylchloroformate, DCC/HOBT etc., could also increase the yield of coupling reactions but these are expensive.

Epoxidation of CPX and CXP Systems in the Presence of PASCOS-

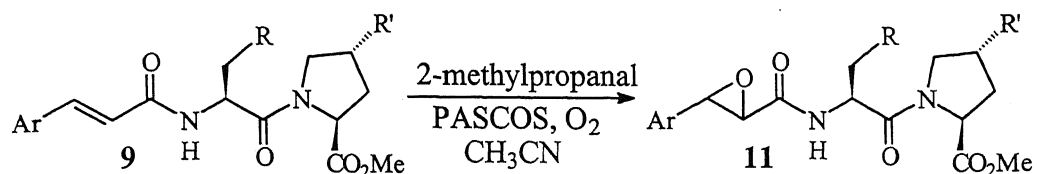
The CPX and CXP systems thus synthesized were subject to epoxidation in acetonitrile in the presence of catalytic amounts (~5 - 10mg) of polyaniline supported cobalt (II) salen and 2-methylpropanal in oxygen atmosphere. Typically, N-cinnamoyl-dipeptide was dissolved in acetonitrile and PASCOS was added to it, followed by the addition of 2-3 equivalents of freshly distilled 2-methylpropanal. The reaction mixture was flushed with oxygen through an oxygen-containing balloon and the contents were allowed to stir for 10-12 h under oxygen atmosphere at ambient temperature. After which, a second lot of 2-3 equivalents of the 2-methylpropanal and the catalyst were added. At the end of reaction (TLC), filtration of the catalyst on a filter paper and base workup of the concentrated residue, followed by water and brine wash resulted in excellent yields (80-90%) of the crude epoxides in high purity (~95-100% - HPLC).



General Scheme For The PASCOS Catalyzed Epoxidation Of CPX Systems

Along with the epoxides, we observed the formation of very minor traces (~5 %) of high polar impurities, which were sparingly soluble in organic solvents like carbontetrachloride, ethylacetate and dichloromethane. No efforts were made to purify or

characterise these dark coloured side products. However, it is our assumption that these could be oligopeptides, formed by polymerization during the epoxidation reactions.



General Scheme For The PASCOS Catalyzed Epoxidation Of CXP Systems

Table-1: PASCOS Catalysed Epoxidation of CPX Peptide Systems

| Entry | CPX Substrate | Epoxide (Yield%) (3S,2R):(3R,2S) ^b | Purity ^a , [α] _D ^{25 °C} |
|-------|---------------|--------------------------------------------------|------------------------------------------------------------|
| 5a | | | 100% |
| | | (73:27) | -124 |
| 5b | | | 96% |
| | | (70:30) | -57 |
| 5c | | | 98% |
| | | (60:40) | -15 |
| 5d | | | 96% |
| | | (75:25) | -72 |

a-determined by HPLC; b-¹H NMR

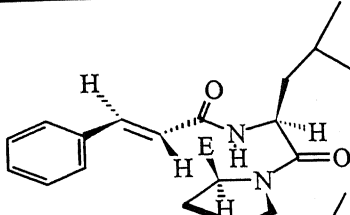
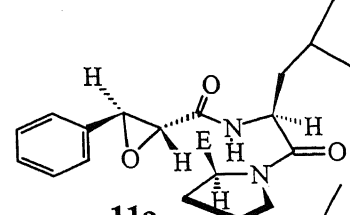
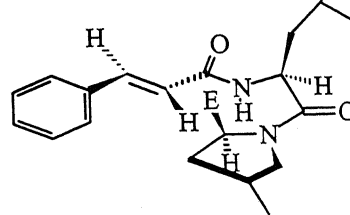
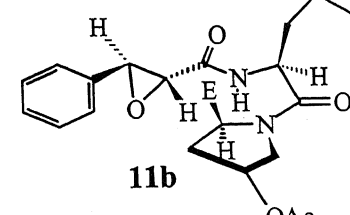
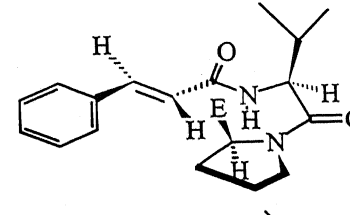
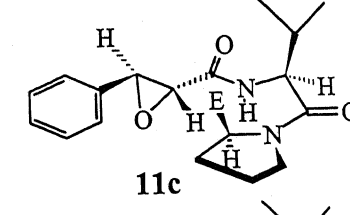
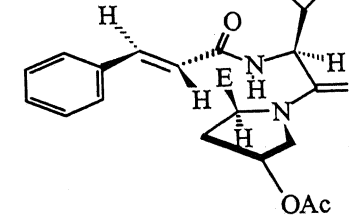
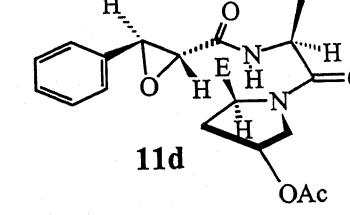
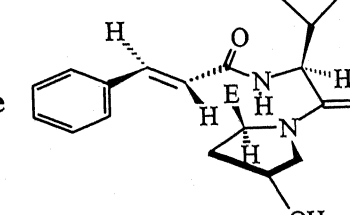
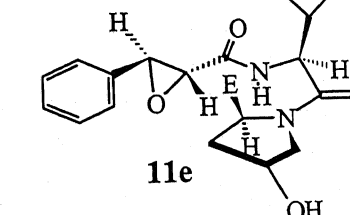
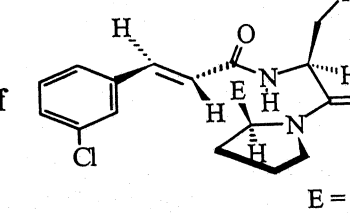
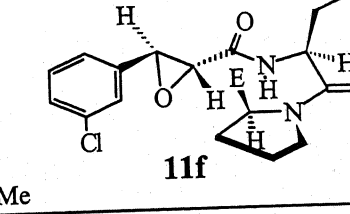
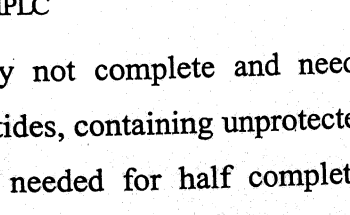
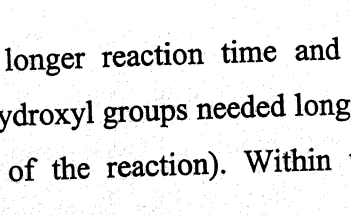

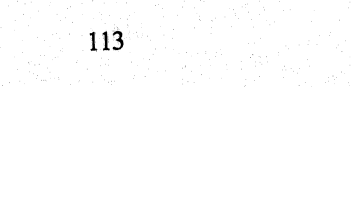


In almost all cases, the color of the reaction mixture turned from colorless in the beginning, to reddish brown after 6-8 h, and finally to pale yellow color at the completion of reaction. Although this change in color was not taken for confirmation of the

completion of reaction, in most cases than not, this was an indication for the formation of required epoxide in near complete proportions.

Glass TLC plates were used and found to be good enough to monitor the reaction proceedings. TLC of the epoxidation reactions were visualized either in iodine chamber or under U.V. light. In general, N-cinnamoyl-dipeptides showed up as very bright spots under U.V. light even at very low concentrations; on the other hand, spots of pure epoxides were hardly visible under U.V. light (254 nm). Completion of reactions were thus, best detected by visualizing under U.V. light, with fluorescent silica gel as the immobile phase. The R_f values of epoxides of CPX peptide systems on TLC plates coated with silicagel G were lower than that of their corresponding olefins (TLC - silicagel G; solvent - EtOAc:Hexane - 2:3 / 1:1). On the other hand, in the case of epoxidation of all the CXP systems, the R_f values of epoxides were higher than that of their olefinic precursors (TLC - silicagel G; solvent - EtOAc:Hexane - 2:3 / 4:1).

The optimum temperature for best performances of these epoxidation reactions was found to be between 25 - 35° C. At temperatures below this range ($< \sim 20^\circ \text{C}$), frustratingly long reaction times of up to 50 - 60 h were needed for the completion of reaction along with the need for more catalyst and the aldehyde. However, time was not the problem on exceeding the temperature range. Yields of epoxidation were observed to be lower than otherwise, at higher temperatures ($> \sim 40^\circ \text{C}$) than the mentioned range. Also, the reaction mixture was messed with significant amounts of high polar impurities. The purity of heterogeneous catalyst was very important. The polymer bound catalyst must be washed sufficiently with acetonitrile until the wash was colorless and dried well before use (please refer to the general procedure for the synthesis of PASCOS catalyst). Presence of traces of unwashed impurities in the catalyst completely inhibited the formation of epoxides. In general, ~ 5 -6 equivalents of isobutyraldehyde was needed for the completion of reaction. Addition of isobutyraldehyde was best done in two lots of 2-3 equivalents each. Addition of the aldehyde in other proportions was less effective. Addition of greater equivalents led to the formation of visible proportions of side products, which were mostly opened products of epoxide by isobutyric acid. Almost all substrates underwent complete epoxidations in identical times at the optimum temperature range. Epoxidations best occurred at a reaction scale of 3-4 mmol (~ 1 -1.2 gm) beyond which, conversions were

Table-2: PASCOS Catalysed Epoxidation of CXP Peptide Systems

| Entry | CXP Substrate | Epoxide (Yield %) | Purity 25°C [α] _D |
|-------|-------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|---------------------------------------------|
| 9a |  |  | 95% |
| 9b |  |  | -54 |
| 9c |  |  | 98% |
| 9d |  |  | -124 |
| 9e |  |  | 90% |
| 9f |  |  | -57 |
| 9g |  |  | 96% |
| 9h |  |  | - |
| 9i |  |  | - |
| 9j | | | 100% |
| 9k | | | -63 |

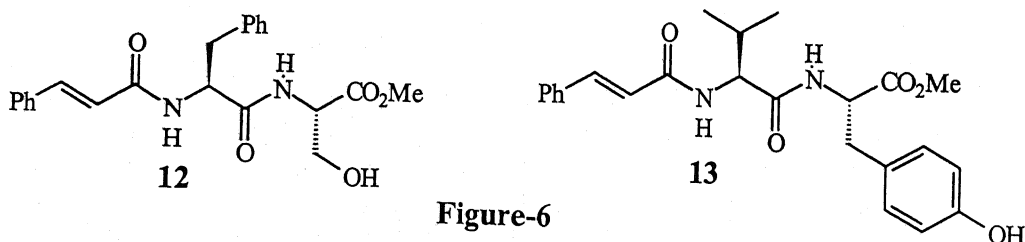
a - HPLC

usually not complete and needed longer reaction time and reagents. N-cinnamoyl-dipeptides, containing unprotected hydroxyl groups needed longer half-times for reaction (time needed for half completion of the reaction). Within the range of mentioned

reaction-conditions the epoxidation of N-cinnamoyl-dipeptides proceeded smoothly to complete conversion in 20 - 24 h to the corresponding epoxides, in excellent yields.

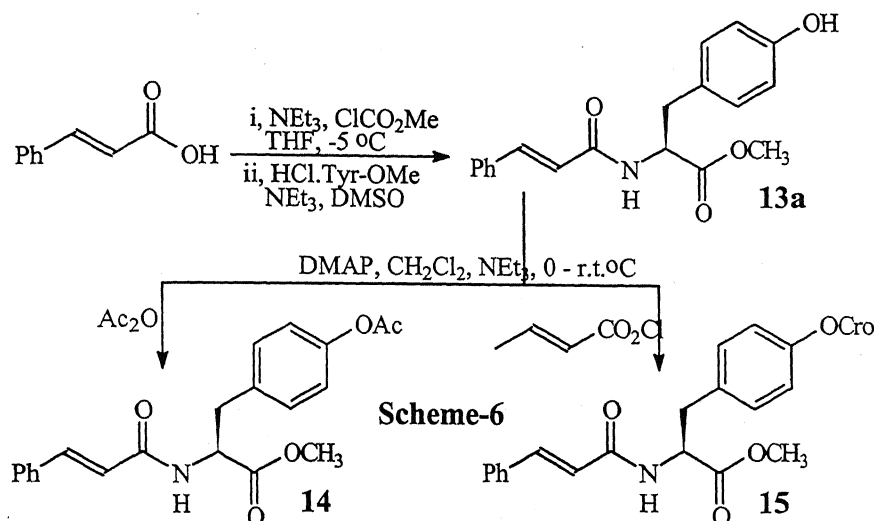
Thus the epoxidation of the CPX peptide substrates (Figure-4) *viz-a-viz* Methyl-N-cinnamoyl-L-proline-L-leucinate, -L-isoleucinate, -L-aspartate and -L-phenylalaninate proceeded smoothly to provide the corresponding epoxides in excellent yields (Table-1). Similarly, epoxidation of the CXP peptide substrates (Figure-5) *viz-a-viz* methyl-N-cinnamoyl-L-leucine-L-prolinate, methyl-N-cinnamoyl-L-leucine-(4-*trans*-oxyacetyl)-L-prolinate, methyl-N-cinnamoyl-L-valine-L-prolinate, methyl-N-cinnamoyl-L-valine-(4-*trans*-oxyacetyl)-L-prolinate, methyl-N-cinnamoyl-L-valine-(4-*trans*-hydroxy)-L-prolinate and methyl-N-(*meta*-chlorocinnamoyl)-L-phenylalanine-L-prolinate proceeded smoothly to provide the corresponding epoxides in good yields (Table-2). In general, the epoxidation reactions on CPX systems needed shorter reaction times (~18-20 h) for complete conversion of the olefinic precursors than the CXP systems (~20-25 h). However, the yields of epoxidation of hydroxyl-protected N-cinnamoyl dipeptide was significantly lower than normally observed for the other systems.

N-cinnamoyl dipeptides with amino acid residues bearing hydroxy side chains in *i*+1 & *i*+2 (Figure-6) positions from the cinnamoyl group were synthesized by the mixed anhydride coupling protocol. Owing to poor solubility in acetonitrile, tyrosine and serine containing N-cinnamoyl-dipeptides, did not undergo conversion to the corresponding epoxides, in these conditions.

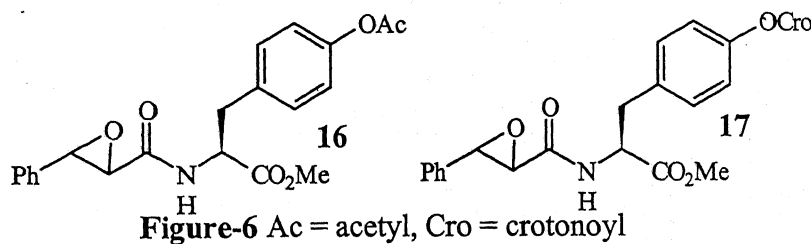


Thus, the N-cinnamoyl dipeptides methyl-N-cinnamoyl-(L)-phenylalanine-(L)-serinate and Methyl-N-cinnamoyl-(L)-valine-(L)-tyrosinate failed to get epoxidised in the above conditions due to poor solubility. Similar was the case with N-cinnamoyl-tyrosinate and the corresponding allyl ether.

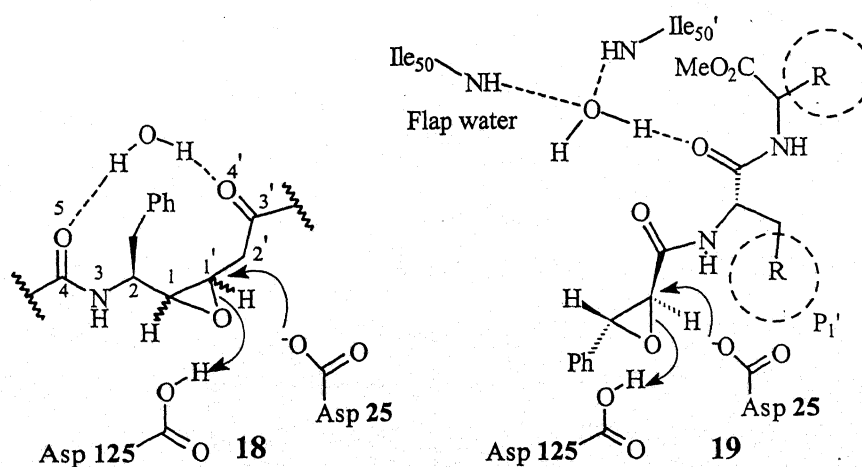
However, we found that hydroxyl protected N-cinnamoyl-L-tyrocinat, underwent epoxidation, to the corresponding epoxide, in good yields. Thus, N-cinnamoyl-tyrosinate was synthesised from cinnamic acid and tyrosine methylester hydrochloride, using the mixed anhydride procedure, as described for the earlier cases. O-acetylation was effected



by reacting the peptide with acetic anhydride in dichloromethane, in the presence of DMAP; and O-crotonoylation, by the reaction with crotonoyl chloride and triethylamine, in dichloromethane in the presence of DMAP at 0 °C. Subjection of methyl-N-cinnamoyl-(O-acetyl)-L-tyrosinate and methyl-N-cinnamoyl-(O-crotonoyl)-L-tyrosinate to epoxidation conditions as above in the presence of PASCOS, yielded the corresponding epoxides in moderate yields.



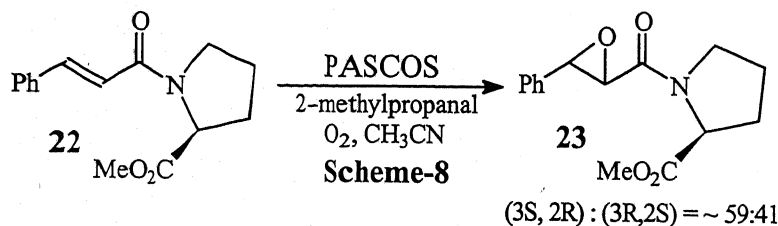
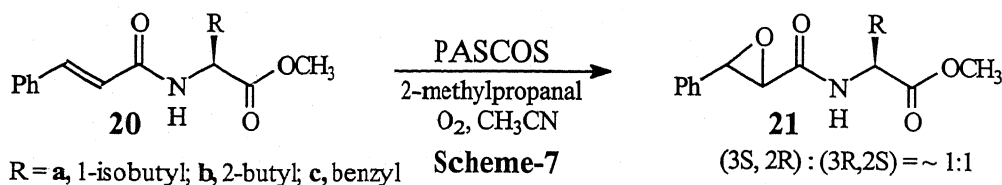
Thus, we have synthesized a library of small molecule N-cinnamoyl pyrrolidine



containing tripeptides, incorporating the trans epoxide, as peptidomimics for recognition at the HIV PR active site. We propose that these epoxides could be good leads as irreversible inhibitors of aspartyl proteases, based on the model proposed by Meek *et al.* We propose that the carbonyl of the P₁' residue might just be conveniently placed to displace the flap water molecule at the HIV PR active site and as a result suitably place the epoxy group between the active-site aspartate carboxyl groups for alkylation, leading to irreversible binding with the protease (Figure-7).

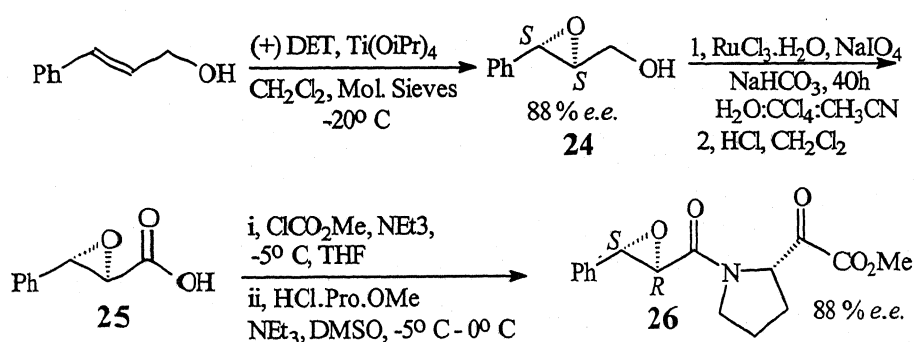
Facial Selective Epoxidation

The presence of a polymer bound catalyst and chiral centers (at least two) in the α,β -unsaturated substrates (N-cinnamoyl-dipeptides) can be expected to impart facial selectivity during the epoxidation of these substrates. The high rotation values for most of the epoxides of CPX systems complimented this thought. Whereas the rotation of the epoxide of methyl-N-cinnamoyl-L-leucinate, -L-phenylalaninate or -L-isoleucinate, synthesized in the presence of PASCOS was quite close to none, the epoxide of methyl-N-cinnamoyl-L-proline showed a rotation of -58° (CH_2Cl_2 , $C=0.001$) synthesized *via*



the PASCOS catalysed protocol. Hence, we reasoned that proline effected a facial bias on the cinnamoyl double bond during its epoxidation. There were two doublets corresponding to each of the epoxy methine proton signals of **21**, but the presence of two pairs of doublets for each of the epoxy methines in the ^1H NMR (δ 4.14, 4.08, 3.99 & 3.97 - β -methine), in the ratio of ~3:2, in the case of **23** intrigued us. In order to ascertain whether this was due to the known modes of cis- and trans-

conformational existence of proline for each of the two possible diastereomers of epoxide, we synthesized (Scheme-9) the methyl- (2R,3S)-epoxy-cinnamoyl-L-prolinate from (2R,3S)-phenylglycidic acid, synthesized by RuCl_3 catalysed oxidation of the (2S,3S)-epoxide of *trans*-cinnamyl alcohol ($[\alpha]_D^{25} = -49$; $C=0.002$, CH_2Cl_2) (88% *e.e.*) which was synthesized by the Sharpless epoxidation procedure in the presence of natural (+)-DET and *t*-butyl hydroperoxide under Sharpless epoxidation conditions.²²³ Thus, 3-phenyl-(2S,3S)-epoxy-1-propanol was oxidised to the corresponding (2R,3S)-glycidic acid in the presence of $\text{RuCl}_3 \cdot \text{XH}_2\text{O}$ and NaIO_4 .²²⁴ The glycidic acid was coupled with methyl-L-prolinate hydrochloride by the mixed anhydride procedure in THF - DMSO with isobutyl chloroformate as the carboxyl activating group to obtain the methyl- (2R,3S)-(3-phenylglycyl)-L-prolinate ($[\alpha]_D^{25} = -203$, $C=0.002$) (88% *e.e.*, determined with respect to the starting optically pure epoxy alcohol **24**). The presence of a single pair of epoxy-methine (δ 4.07 & 3.99) doublets for each of the methine hydrogens in the ^1H NMR proved that the observed pairs of doublets in the polyaniline supported cobalt catalysed epoxidation product was due to the presence of two diastereomers. However,

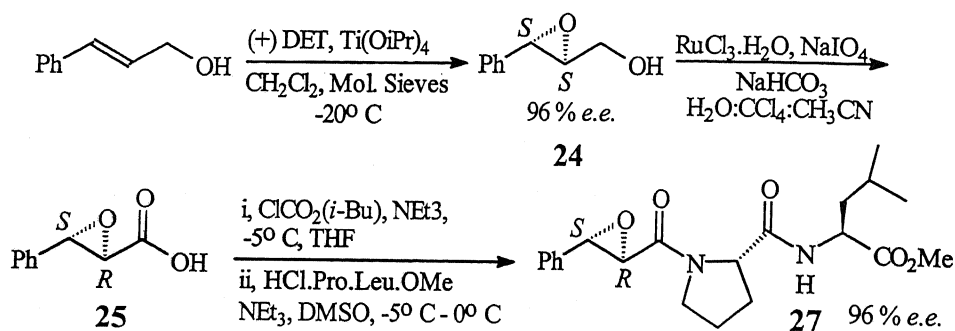


Scheme-9 Methyl-(3S,2R)-N-Glycidyl-Proline

presence of a *pair* and not one *single* doublet for each epoxy methines suggested that they could be because of the *cis*, *trans* modes of conformational existence of proline. The signs of rotation of the pure (2R,3S) epoxy compound, synthesized through the Sharpless epoxidation protocol and that of the mixture of diastereomers, synthesized using the PASCOS catalysed protocol was the same (negative). Implying that the epoxidation had occurred stereoselectively but with a low *e.e* (*e.e.*~20), in the presence of PASCOS.

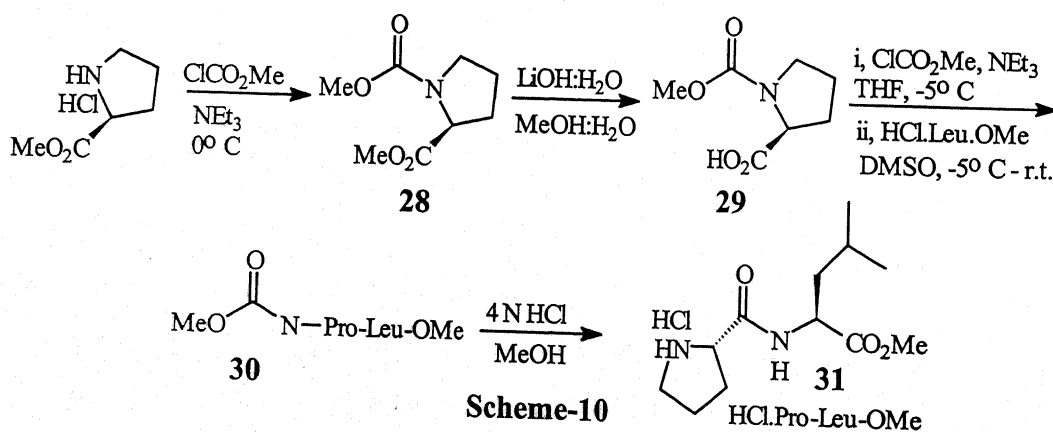
Among the tripeptides, the epoxide of N-cinnamoyl-Pro-Leu was found to have a high value of rotation. The ^1H NMR of this compound also showed two pairs of doublets for

each of the epoxy methines. These pairs were in the ratio 4:1 which, we assumed, could be the diastereomeric ratio; and within each pair, the ratio of the two doublets in each case was ~3:1 which, we reasoned, could be due to the existence of proline in *cis* and *trans* conformations. We wished to confirm this reasoning, by synthesizing the optically pure (3*S*,2*R*) epoxide of methyl-*N*-cinnamoyl-*L*-pro-*L*-leucinate. Thus, we synthesized this epoxide by the Sharpless epoxidation protocol, as described earlier (Scheme-10).



Scheme-10 Synthesis of Methyl-(3*S*,2*R*)-Glycidyl-Pro-Leucinate

The (2*S*,3*S*)-epoxyalcohol was synthesized from *trans*-cinnamyl alcohol by following Sharpless epoxidation protocol.²²³ Oxidation of the epoxyalcohol in presence of RuCl₃·3H₂O and NaIO₄²²⁴ gave the corresponding carboxylic acid. Coupling of the glycidic acid with Methyl-*L*-proline-*L*-leucinate hydrochloride by the mixed anhydride procedure with isobutylchloroformate as the carboxyl activating group gave the optically pure Methyl-*N*-(2*R*,3*S*)glycyl-*L*-proline-*L*-leucinate ([α]_D²⁵ = -191, C = 0.010, CH₂Cl₂).

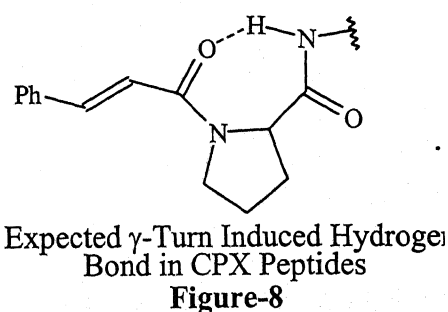


Scheme-10

The presence of a single pair of doublets for each of the epoxy methines in the ¹H NMR of the diastereomerically pure (96 % ee) epoxy tripeptide, corresponding to the major pair of doublets in the epoxide synthesised by the PASCOS protocol, and the similar

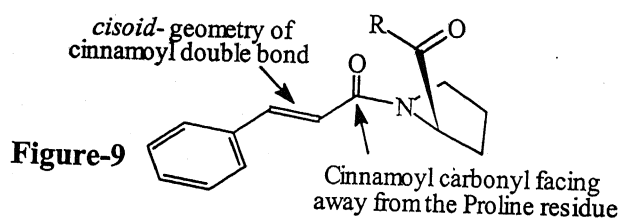
signs of rotation for both the epoxides, implied that the PASCOS catalyzed epoxide was formed predominantly as the (3S,2R) diastereomer. However, presence of a pair of (*again!*) and not a single doublet corresponding to each of the epoxy methines meant that the peptide existed distinctly in two different conformers - probably the *cis* and the *trans* conformers which are well recorded among proline containing systems.²²⁵⁻²²⁷ To substantiate this reasoning that the observed pair of doublets were due to the existence of proline in two distinct conformers, we subjected the epoxides synthesised by the Sharpless and the PASCOS protocols to ¹H NMR studies in solvents of different polarities (Figure-10). It is known that change in solvent polarities lead to change in the conformational population of compounds, especially in the case of peptides. Hence, to a sample of each of the epoxides in CDCl₃ was added (CD₃)₂SO in steps of 0.05 mL and the ¹H NMR spectrum was recorded for each of the samples. The change in ratio of the pairs of doublets (for the epoxy methine proton) on addition of (CD₃)₂SO, indicated indeed, that the observed pair of signals corresponded to two distinct conformers (probably the *cis* and *trans* conformers) of the peptide which were in distinct populations in different solvent mixtures. In solution, each diastereomer seems to equilibrate into the *cis* and *trans* conformers.

Substrate Directed Facial Selectivity



We had initially assumed that the observed facial selectivities during epoxidation of CPX systems could be arising due to a semi-rigidified secondary structure of the peptide system, induced by a γ -turn hydrogen bond, between the carbonyl of the cinnamoyl group and the amide NH of the *i*+2th amino acid residue. However, NMR studies on the CPX systems at different solvent polarities showed the absence of such a hydrogen bonding interaction.

An inspection of the minimum energy conformers of these CPX peptide systems (energy minimizations were achieved with the help of Hyperchem Computer modelling software, optimized with MM, optimized with MM⁺), revealed that the amino acid X of the CPX systems were suitably placed to cloud one face of the cinnamoyl double bond, by virtue of the proline moiety. The cinnamoyl group preferred to adopt a *cis*-conformation with its carbonyl facing away from the proline residue and the cinnamoyl double bond preferred a *cisoid*-conformation, with respect to the cinnamoyl carbonyl (Figure-9). The free energy of CPX peptides with the proline adopting a *trans* conformation was found to be higher than that for the *cis* conformer, by an order of ~4-5 kcal/mol, determined by Hyperchem energy minimization studies.



All these structural elements, seem to induce the observed facial bias during the epoxidation of the cinnamoyl double bond of CPX systems, in the presence of PASCOS catalyst. This facial bias resulted in the formation of (3*S*,2*R*)-glycidate-peptides, in high diastereomeric purity (~8 : 1).

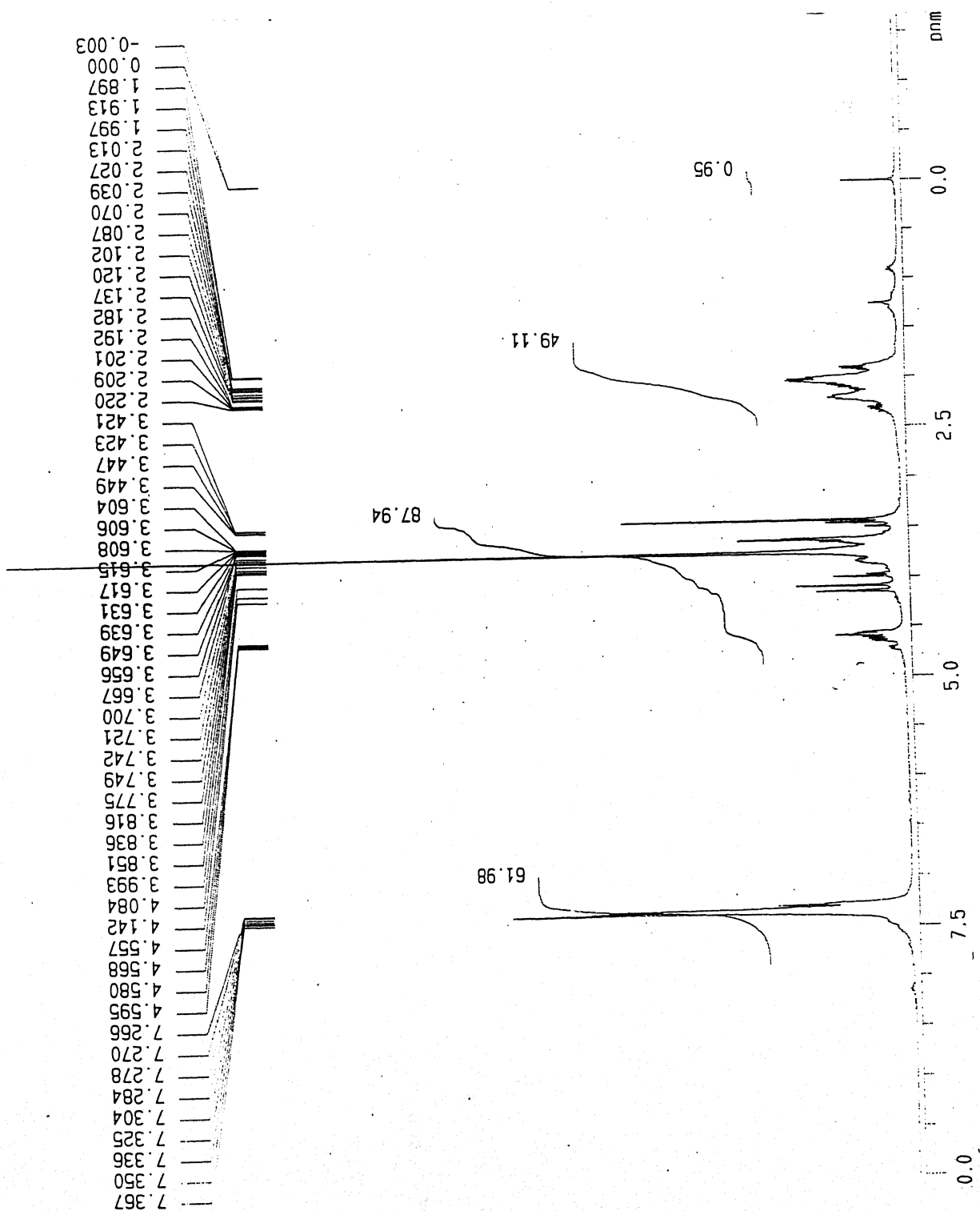
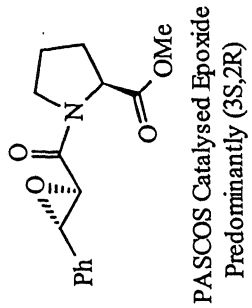
However, similar facial-selectivities were not observed for the epoxidation of CXP systems, which was reflected in their low rotation values and in the appearance of two pairs of doublets for epoxy methine protons in equal ratios, in their ¹H NMR spectra. Inspecting the minimum energy conformers of the CXP peptide substrates using the Hyperchem molecular modelling package revealed that the secondary structures adopted by these systems do not impart any significant facial bias to the cinnamoyl double bond (Figure-11).

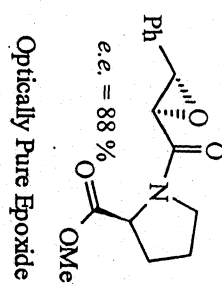
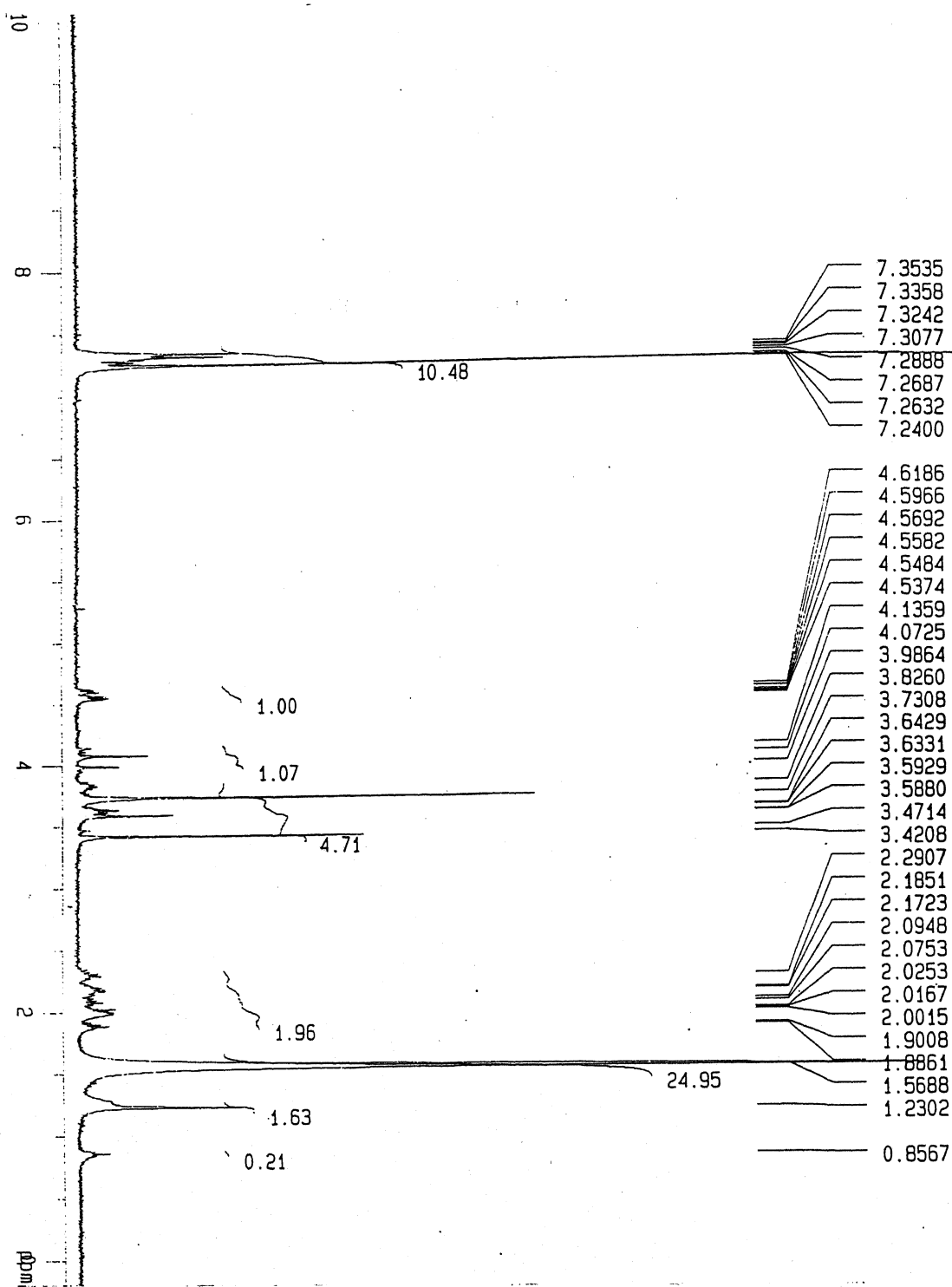
The epoxide from methyl-N-cinnamoyl-L-leucinate synthesized through the PASCOS catalysed protocol, showed near-zero rotation and pairs of doublets for the epoxy methines, of equal intensities. Similar was the case with the epoxide from methyl-N-cinnamoyl-L-leucine-L-prolinate. However, the rotation value of the epoxide of methyl-acetyl-N-cinnamoyl-L-leucine-(4-*trans*-oxyacetyl)-L-prolinate, was quite high! An

examination of the minimum energy conformation of the latter peptide revealed that the observed facial bias was due to the presence of oxyacetyl group on proline, which was suitably placed to cloud the cinnamoyl double bond, inducing facial bias - a feature absent among other CXP systems.

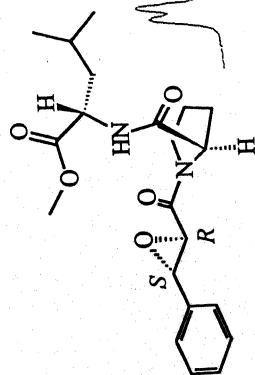
Conclusion

Thus, we have developed a highly efficient and cost effective methodology for the synthesis of tripeptides, incorporating *trans* oxirane adjacent to the scissile peptide bond, as elements for irreversible binding with protease active site. We have observed that N-cinnamoyl-Proline containing peptides undergo epoxidation in the presence of PASCOS and 2-methylpropanal to yield the corresponding epoxides in high diastereomeric ratios and yields. The stereochemistry of the predominant epoxide diastereomer has been determined by chemical correlation, to be (3S,2R)-3-phenyl-glycidyl-peptide. These peptides seem to exist in two distinct, *cis* and *trans* conformers. Molecular modelling studies on the olefinic precursors suggested the effect of proline in positioning the second amino acid residue (from the cinnamoyl group) so as to create the observed facial bias on the cinnamoyl double bond.





CPLE-1H



PASCOS CATALYZED
PREDOMINANT DIASTEREOMER
 $[\alpha]_D^{25} = -124^\circ$ (CH_2Cl_2)

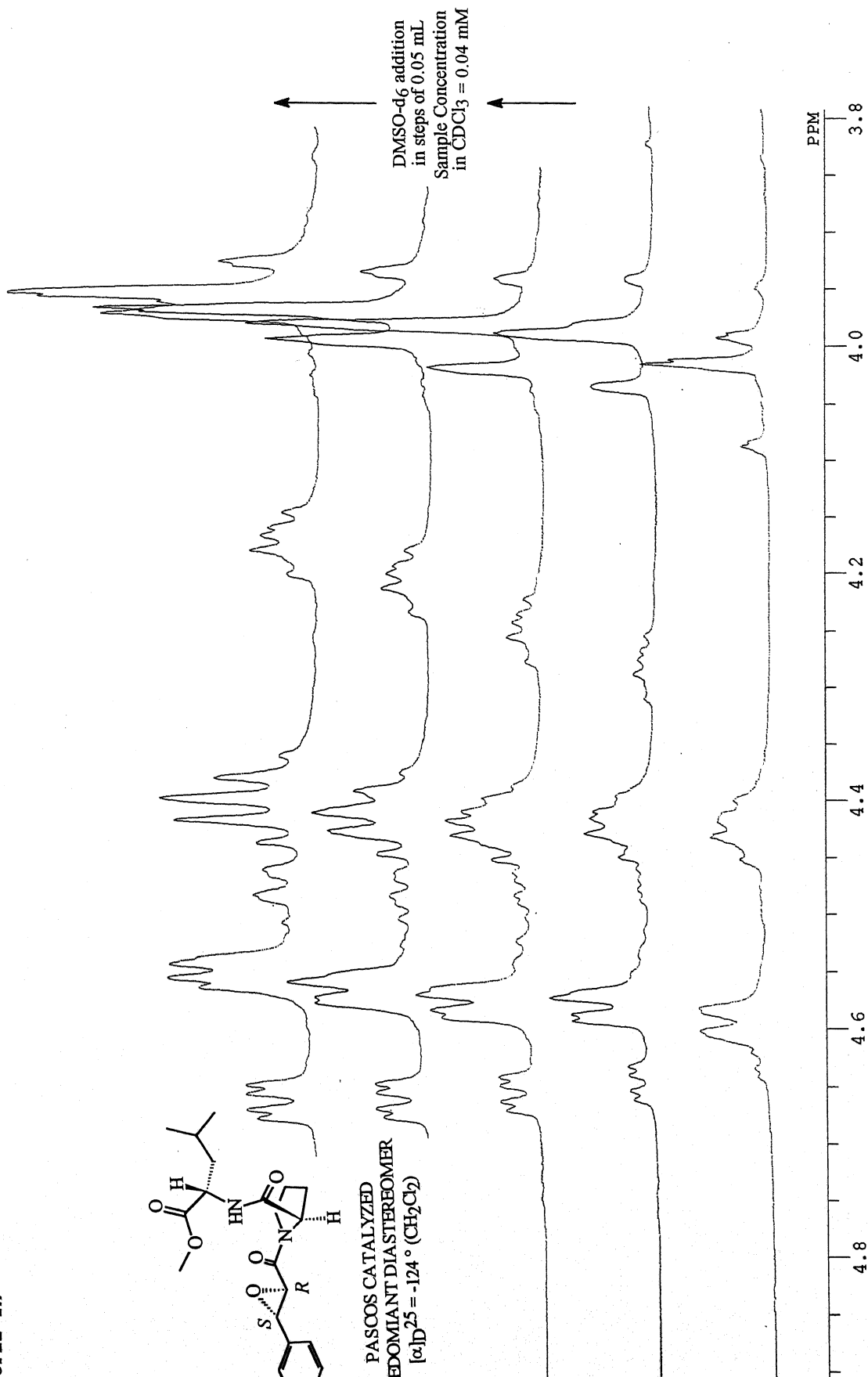
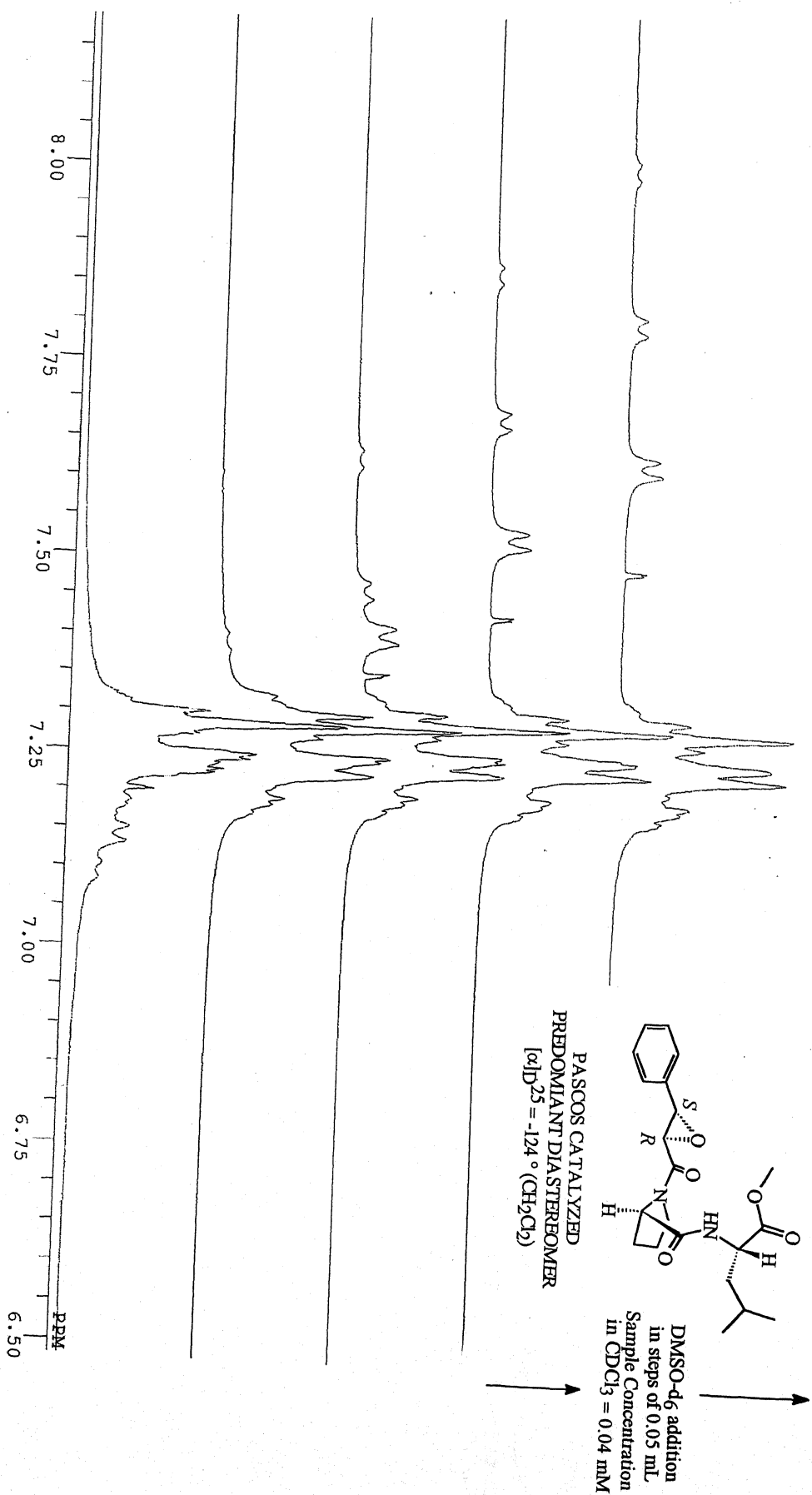


FIGURE -10



CPL-1H

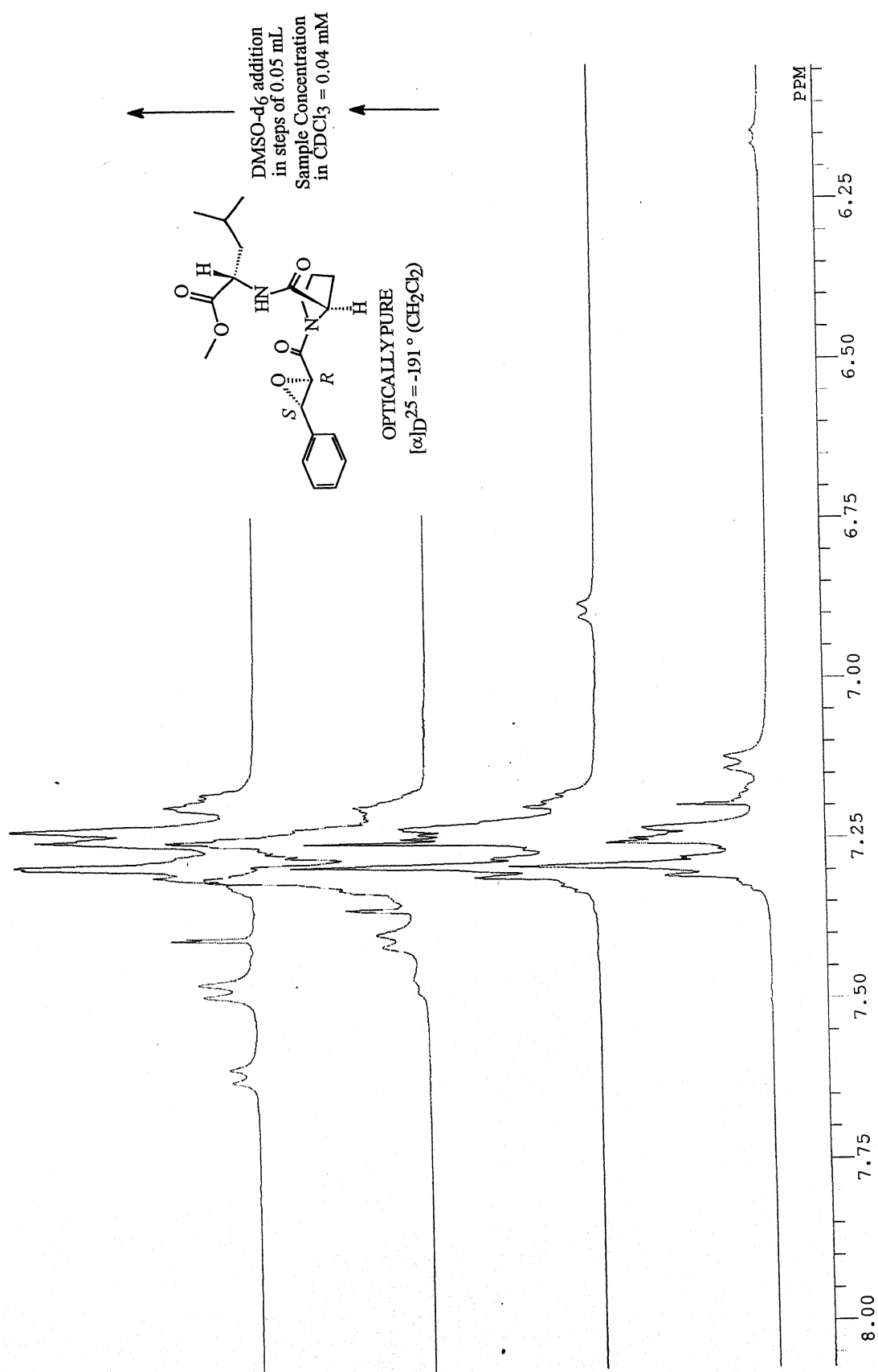
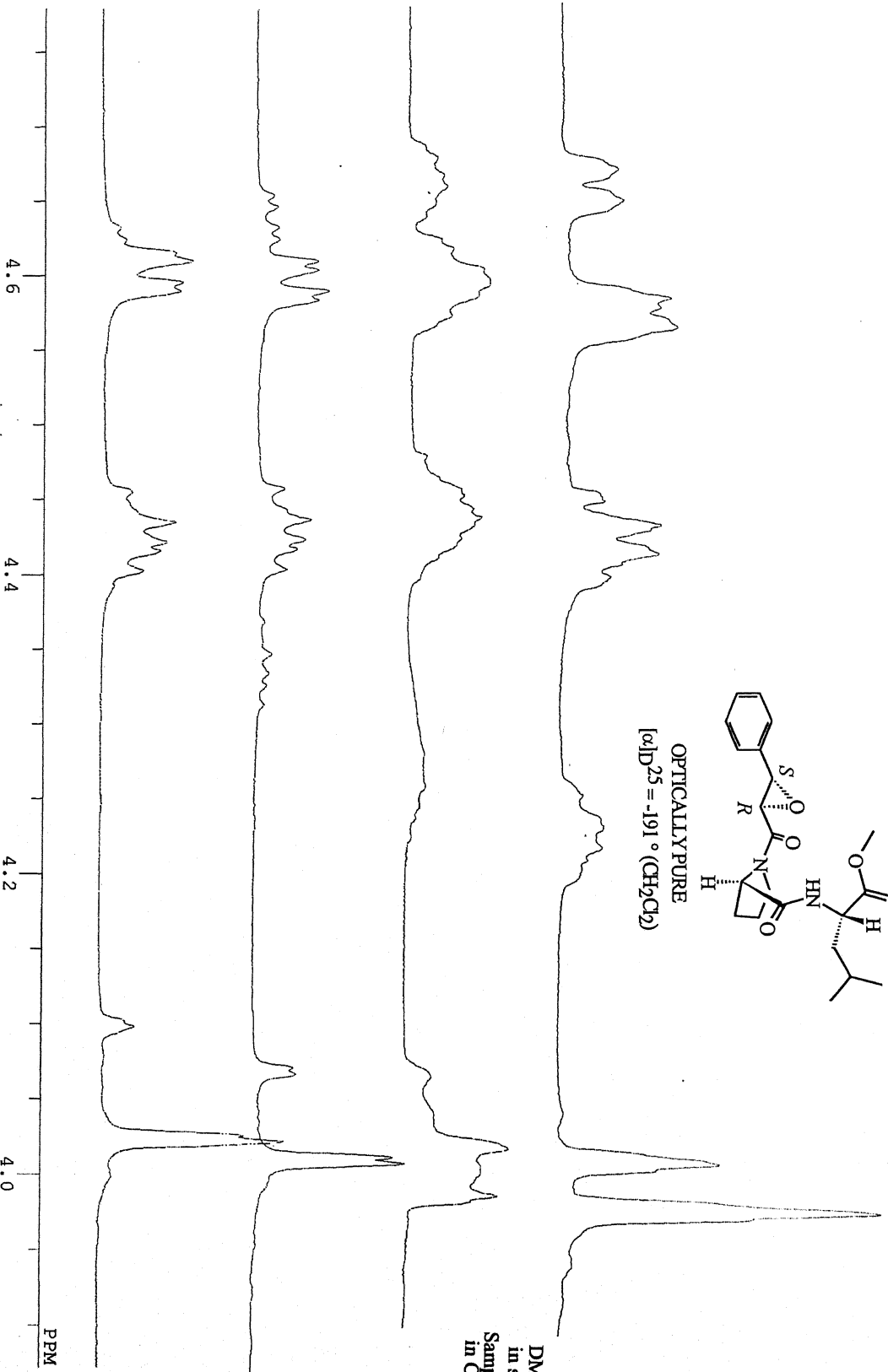
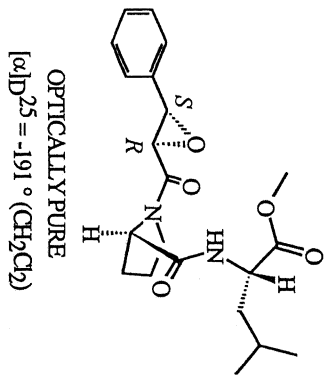
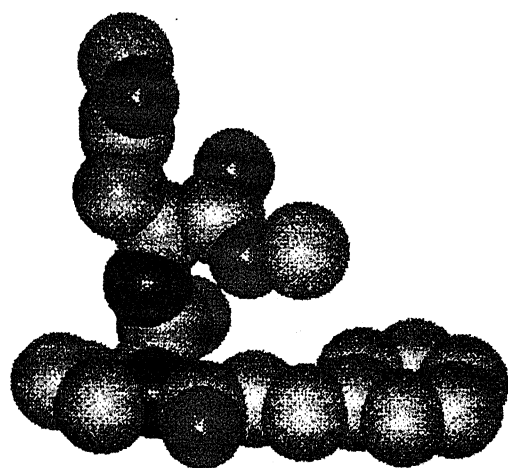


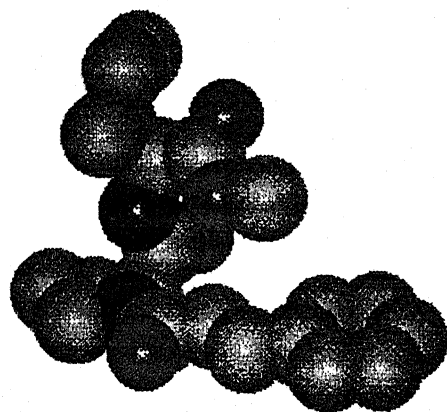
FIGURE-10



DMSO-d₆ addition
 in steps of 0.05 mL
 Sample Concentration
 in $\text{CDCl}_3 = 0.04 \text{ mM}$



Methyl-N-Cinnamoyl-proline-aspartate



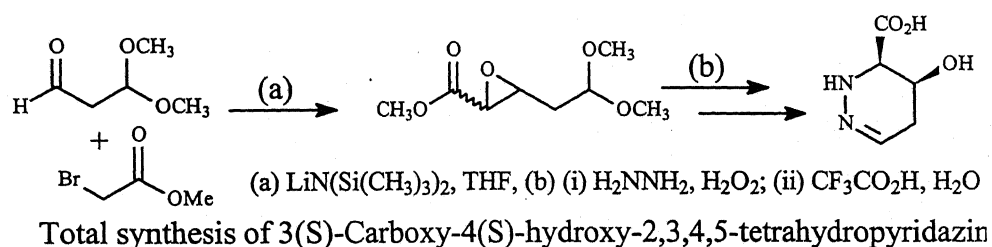
Methyl-N-Cinnamoyl-proline-isoleucinate

FIGURE-11

Introduction to Statine Analogues

An increasingly popular approach to the development of therapeutic agents involves the design of compounds that closely mimic natural peptide hormones or substrates. Generally this entails reducing the natural peptide to a minimally required size and replacing normal amino acids and peptide regions with synthetic analogues that confer desired properties such as enhanced binding, hydrolytic stability or antagonism versus agonism.²²⁸ Recent works²²⁹⁻²³⁸ have shown that peptides in which the scissile bond is replaced by a transition-state analogue provide a useful starting point in the design of tight-binding inhibitors of the HIV-PR.

New non-standard aminoacids, which might serve to replace normal amino acids are often synthesized or discovered (in natural products). Such compounds offer possible therapeutic utility. Luzopeptin, a dimeric cyclic depsipeptide is a natural product that interacts with DNA and plays a role in its activity. It contains two unique aminoacid constituents.²²⁸ One is a quinoline (1) and the other is 2(s)-carboxy-3(s)-hydroxy-2,3,4,5-tetrahydropyridazine (2).



Scheme-1

Unnatural β -amino acid statine and its analogues, like isostatine and dolaisoleucine, have been known to impart high binding potencies to peptide systems of which they form a part, through hydrogen bonding interactions between their hydroxyl functional group and the aspartyl groups of aspartyl proteases.

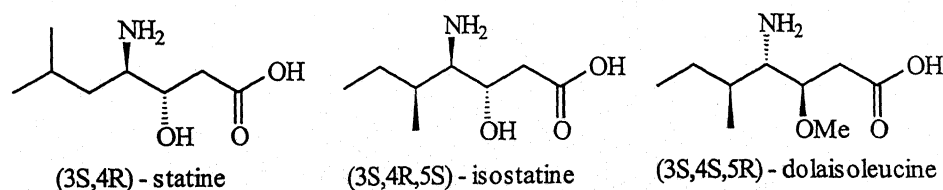
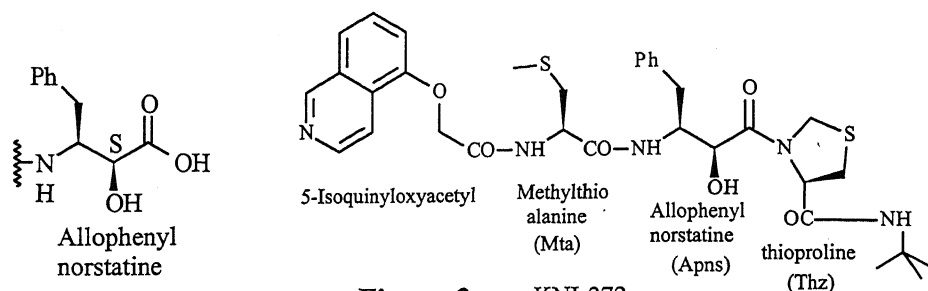


Figure-1

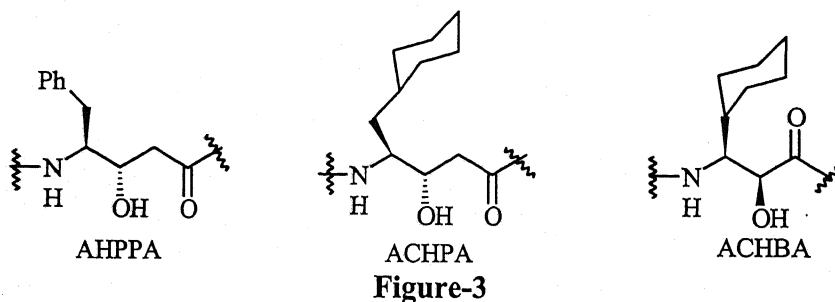
Design of isostructural statine analogues for the synthesis of HIV PR inhibitors have required the replacement of the isobutyl and 2-butyl side chain groups with larger aromatic and cyclohexyl groups, for positive complimentary binding with hydrophobic

pockets at the enzyme active site. Several unnatural statine acid derivatives, incorporating required isosteres to serve as appropriate peptidomimetics for inhibition of HIV-PR have been reported. The most notable among them being the AHPBA (allophenylnorstatine) amino acid

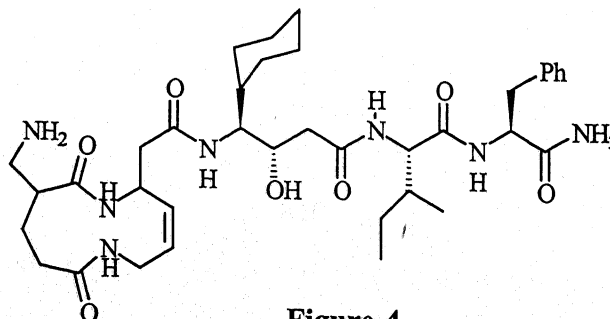


((2S,3S)-3-amino-2-hydroxy-4-phenylbutyric acid), designed by Kiso *et al*, incorporating the α -hydroxycarbonyl isostere as transition state peptidomimic of the Phe-Pro scissile site in HIV PR substrate.^{239,240,241} Incorporation of AHPBA into -Asn-Phe-Pro- peptide and further lead optimizations on the tripeptide have led to the discovery of kinostatin-272 and -227, two HIV1-PR inhibitors of picomolar potency.

AHPPA ((3S,4S)-4-amino-3hydroxy-5-phenylpentanoic acid) is a homologue of AHPBA and has also been found to lead to potent HIV1-PR inhibitors when incorporated as part of tripeptide systems.^{242, 243}



Modifications of phenyl to cyclohexyl substituent in AHPBA and AHPPA and further



homologations have led to the design of yet another class of such statine acids, ACHPA ((3S,4S)-4-amino-3-hydroxy-5-cyclohexylpentanoic acid) and ACHBA ((3S,4S)-4-amino-3-hydroxy-4-cyclohexylbutanoic acid). Novel high potent inhibitors have been reported²⁴², incorporating these amino acids (Figure-4, 5).

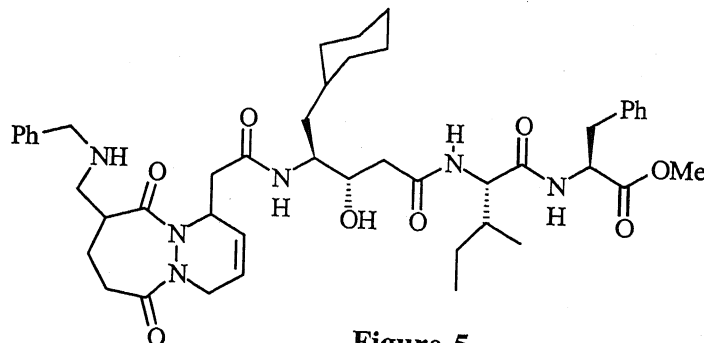


Figure-5

One of the most potent HIV1-PR inhibitors to-date, already in its advanced stage of clinical trials for this cause, Ro 31-8959 (Figure-6) incorporates in itself, the reduced version ((2R,3S)-1,3-diamino-2-hydroxy-4-phenylbutane) of AHPBA as a Phe-Pro peptidomimic.²⁴⁴

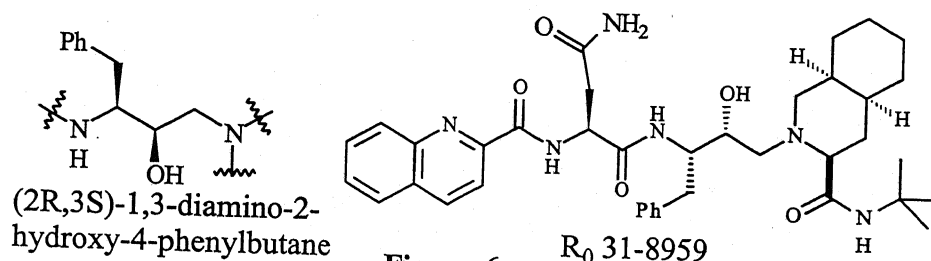
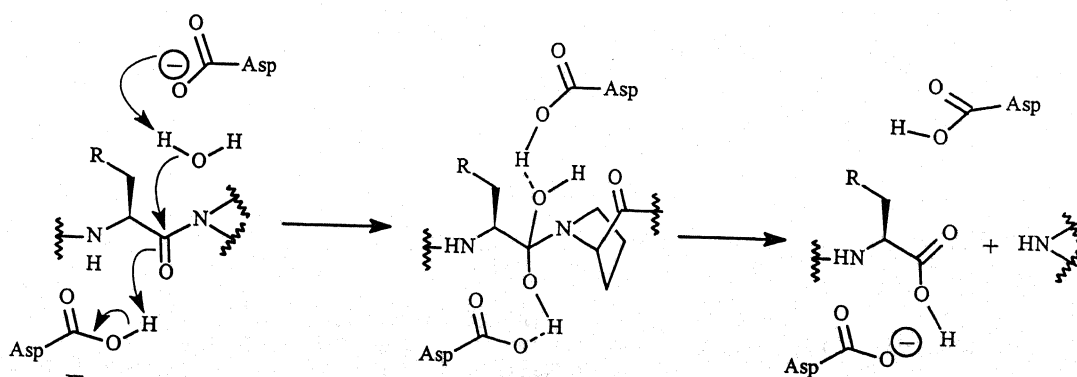


Figure-6

All the statine acid analogues are based on the transition state mimicry of amide hydrolysis (Figure-7) by an aspartyl protease.



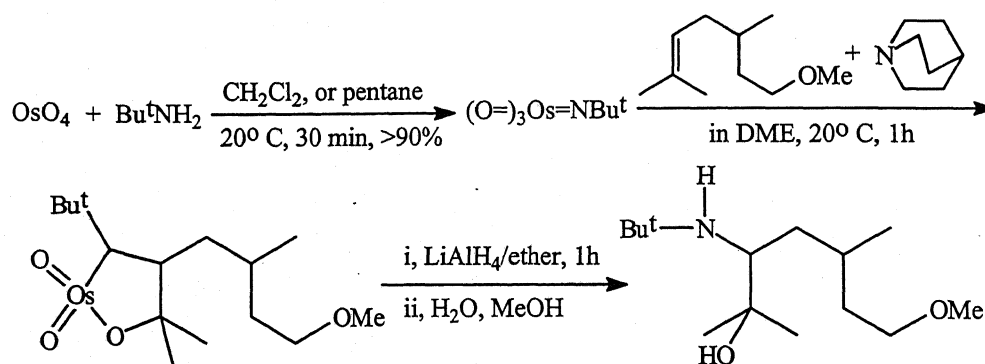
Enzyme reaction mechanism of HIV PR at the Phe-Pro Scissile amide bond site

Figure-7

The hydrogen bonds between the aspartic acid carbonyl groups of protease and the hydroxyl groups of the substrate transition state are significant and hence incorporated in the design of these high-binding inhibitors.^{241, 239}

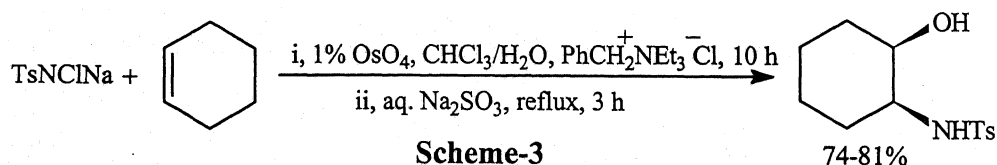
There is a need, thus, for synthetic methodologies than can generate these statine analogues individually, or as part of peptide systems, for the design of potent HIV PR inhibitors. Quite a few methodologies exist, for the synthesis of hydroxyethylamine derivatives. Some of the most attractive and applicable of them are presented below.

In an extension of the *cis* dihydroxylation of alkenes with osmium tetroxide, Sharpless has developed a series of reactions,²⁴⁵⁻²⁴⁹ in which an osmium imine species is added to an alkene, forming a cyclic amide ester, which on reductive work-up gives a *cis*-amino alcohol or its derivative. The nitrogen is delivered to the least substituted end of the C=C bond. It has been recommended that the imine be complexed with quinuclidine (Scheme-2).²⁴⁶



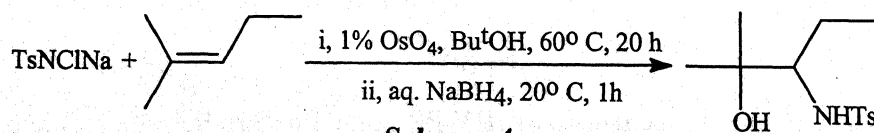
Scheme-2

Two related procedures employing osmium in catalytic quantities and providing a removable nitrogen substituent, have been developed.^{247,248} Scheme-3 represents a procedure suitable for mono- and 1,2-di-substituted alkenes.²⁴⁷



Scheme-3

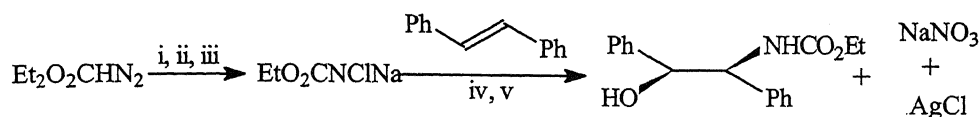
Scheme-4 is suitable for 1,1-di- and tri-substituted alkenes;²⁴⁷



Scheme-4

Scheme-5 is applicable to mono- and 1,2-di-substituted alkenes, especially electron-deficient alkenes²⁴⁸ and can be extended to trisubstituted alkenes by incorporation of

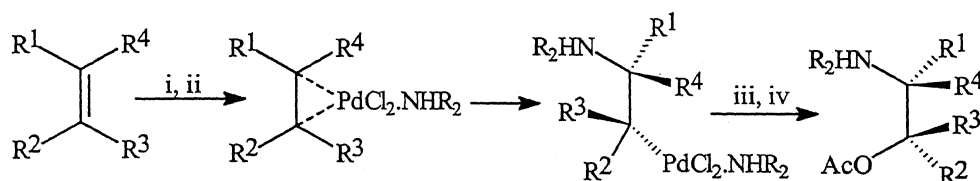
Et₄NOAc in the reaction mixture.²⁴⁹



i, Bu^tOCi/MeOH, 0 °C, 15 min; ii, NaOH/MeOH, 0 °C, 10 min; iii, evaporate to dryness
iv, MeCN, AgNO₃, 20 °C, 5 min; v, 1% OsO₄, aq. Bu^tOH, 18 h, 20° C, then aq. Na₂SO₃, reflux, 3h

Scheme-5

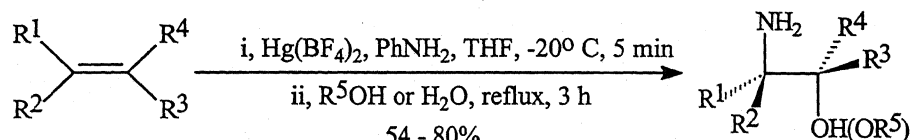
Palladium complexed alkene can be *trans*-opened by an amine nucleophile.^{245,25} Oxidative workup of the resulting σ -palladium species yields an amino alcohol (as its acetate ester). Depalladation occurs with inversion, yielding over all *cis* stereochemistry (Scheme-6).



i, (PhCH)₂PdCl₂, THF, N₂, 0° C, 10 min; ii, R₂NH, -50° C, 50 min;
iii, Pb(OAc)₄/AcOH, -50° C, 5 min, then 20° C, 2 h; iv, KBH₄, 20° C, 20 min

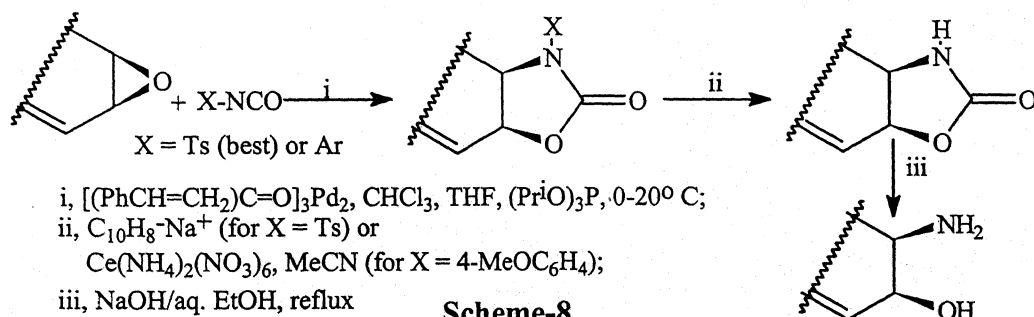
Scheme-6

Related transformations can be achieved using mercury activation to yield *trans* stereospecific product where the mercury itself acts as the oxidant (Scheme-7).²⁵¹



Scheme-7

There are numerous examples^{252, 253, 254} of metal mediated oxyamination of olefins.



Scheme-8

Indirect methods of preparing amino alcohols from alkenes include the *cis* opening of acyclic or cyclic vinyl epoxides with tosyl or aryl isocyanates (Scheme-8)²⁵⁵ and the well-known complementary *trans* opening of epoxides with nitrogen nucleophiles.

Present Study

From the foregone discussions, one can realize the importance of the existence or development of a synthetic methodology for the generation of statine, norstatine, allophenylnorstatine and related analogues, containing the hydroxyethylamine isostere. The advances in high thorough-put screening technologies have paved way for the generation of libraries of small drug like molecules for the discovery and optimization of lead compounds for new drugs in the pharmaceutical industry. Crippled by the immediate non-availability of such high throughput screening techniques against HIV, we focused on the generation of chemically and functionally diverse libraries of small peptides comprising the required binding elements using a polymer-supported technique.

Traditionally, polymer-supported *substrates* upon which chemical reactions are performed, have found wide spread application in the synthesis of various chemical entities. But, these are limited in utility, in terms of the loading capacity of the resin (usually in micro molar scales); availability of appropriate resin of choice; and not the least, their cost. We have sought to address this problem by employing a polymer-supported *catalyst*²⁵⁶ which can promote a reaction on large scale in good yields with optimum use of reagents.

We realized that extension of the polyaniline supported cobalt (II) salen catalyzed epoxidation protocol for the epoxidation of N-cinnamoyl-dipeptides, into the generation of 3-amino-2-hydroxy-3-phenylpropionic acid (AHPPRA - β -phenylisoserine) derivatives, could be realized by the simple opening of the earlier generated epoxides with different amines. Based on the transition state mimetic concept one could expect the β -phenylisoserine derivatives to function as good transition state analogues of the known statine amino acids discussed earlier.

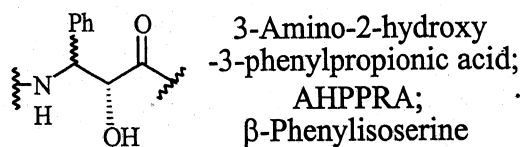


Figure-1

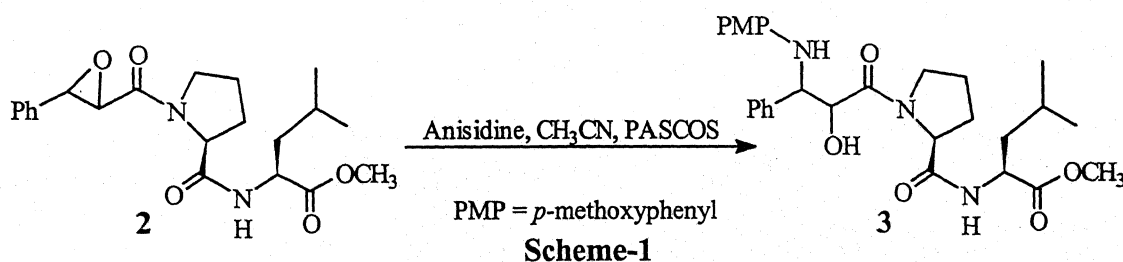
As has been discussed earlier, the optimum chain length for most potent inhibitors of HIV PR have been found to be tripeptides. Thus, we set to synthesize novel class of tripeptides as substrate-based HIV PR inhibitor leads, containing the unnatural amino acid 3-amino-2-hydroxy-3-phenylpropionic acid (AHPPRA) named β -phenylisoserines

(Figure-1), incorporating a hydroxymethylenecarbonyl (HMC) isostere adjacent to the scissile Phe-Pro amide bond.

Eight sites within the HIV-1 gag-pol polyproteins are known cleavage sites for the HIV PR,^{257,258} exhibiting limited primary sequence identities. The cleavage sites are generally characterized by short hydrophobic peptides, flanked by relatively polar sequences. These features are thought to be prominent on the surface of the precursor molecules.²⁵⁹⁻²⁶² Hence, we reasoned that the presence of hydrophobic aromatic rings at the N-terminal with polar substituents like the hydroxyl and methoxy groups in the unnatural amino acid statine analogues would lead to its better binding. This could be achieved by opening of the epoxides with suitable aromatic amines containing hydrophobic and polar substituents like -methyl, -methoxy, -bromo and -hydroxyl groups.

The polyaniline supported cobalt salen catalyzed epoxidation of cinnamoyl-mono-peptides and its subsequent opening with anilines has been reported as preliminary studies, from our group.²⁶³ With the methodology having been successfully extended for the synthesis of epoxides of N-cinnamoyl-dipeptides (discussed earlier) in high enantiomeric purity, we wished to extend this procedure for the general synthesis of tripeptides, containing the β -phenylisoserine derivatives, incorporating the hydroxymethylenecarbonyl isostere, with diversities at the P₁ and P₂ site, thereby making this an attractive protocol for the generation of library of small drug leads in a combinatorial fashion.

Thus, we subjected the epoxide of N-cinnamoyl-L-pro-L-leucinate **2**, generated in the presence of PASCOS catalyst, to opening with anisidine in the presence of the same catalyst, in acetonitrile (Scheme-1). We were gratified to note that opening of the epoxide indeed occurred in good yields, resulting in the β -phenylisoserine derivative containing tripeptide **3**.



However, this reaction would achieve greater importance as a methodology for the synthesis of tripeptides possessing the β -phenylisoserine derivatives, if it could be

After stirring for 12 h, an additional lot of 2-methylpropanal was added and the mixture was stirred for another 8 - 12 h. The oxygen balloon was removed and the aniline (1 equivalent), along with PASCOS catalyst (~10 mg) was added to this mixture and it was further stirred for another 10-15 h.

N-Cinnamoyl-dipeptides **7** Used in the Synthesis of AHPRA Containing Tripeptides **9**

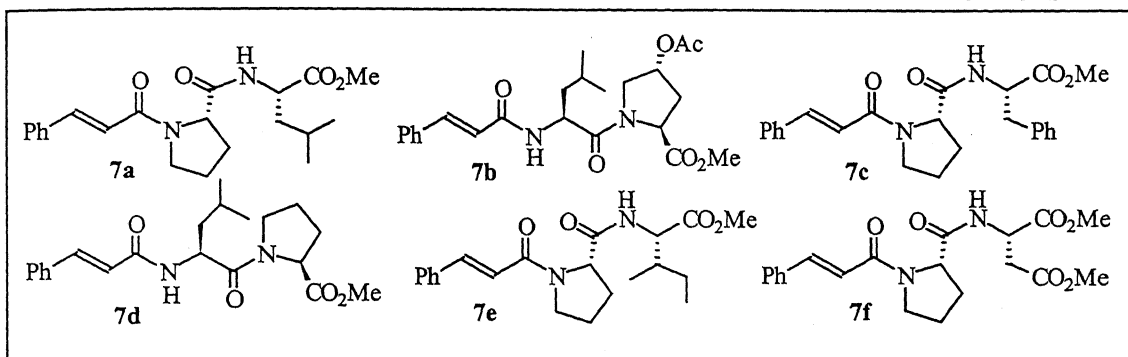


Table-1

Amines **8** used in the synthesis of tripeptides β -Phenylisoserine derived Tripeptides

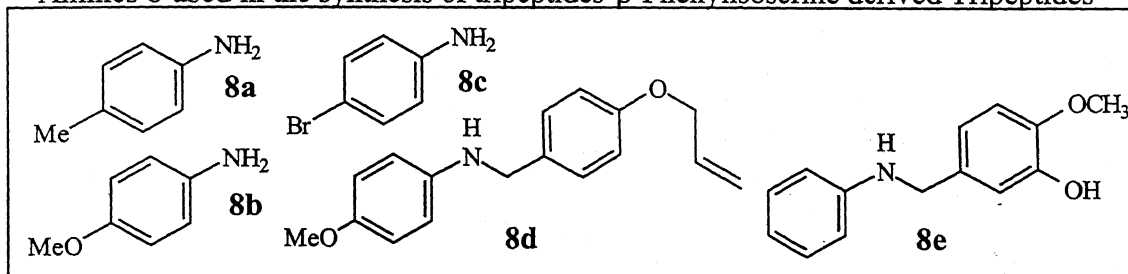
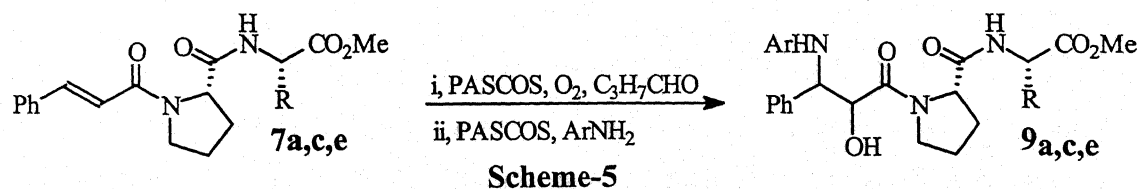
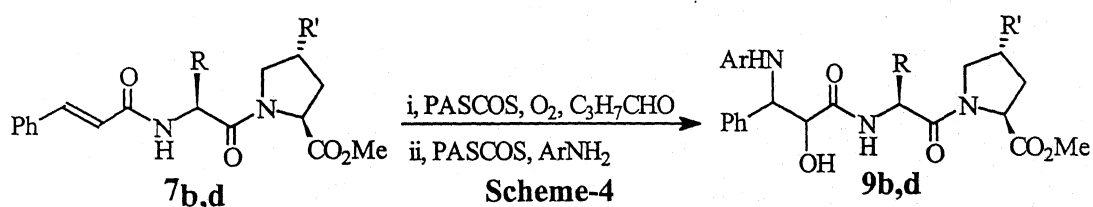


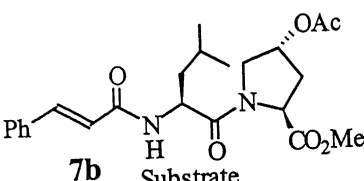
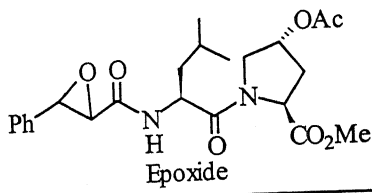
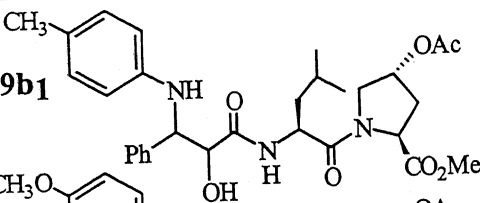
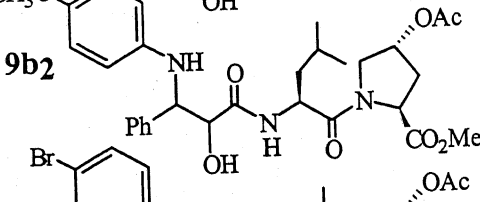
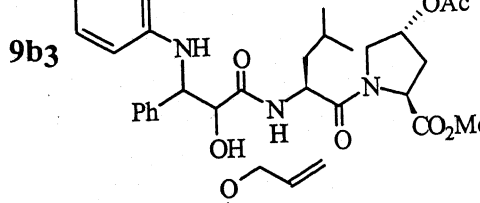
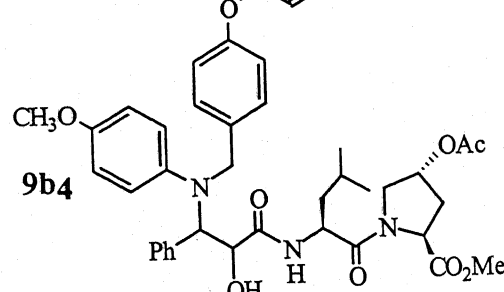
Table-2

The isolation of the resulting tripeptides (most of which are solids) did not require any aqueous workup and column chromatography. The unreacted starting materials were removed during washing with hexane- CCl_4 -EtOAc solvent systems.



The catalyst was filtered and the solvent was removed under vacuum to yield a residue which was taken in minimum volume of EtOAc: CCl_4 and slow drop wise addition of

hexane to the solution, resulted in the precipitation of the crude tripeptide, which was filtered off. Similar repeated washing of the concentrated mother liquor, resulted in complete isolation of the polar tripeptides, containing the β -phenylisoserine derivatives in good over all yields, mostly as solids.

| Product | | Syn : Anti ^a | (Yield %) ^b |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--|-------------------------|------------------------|
| <div style="display: flex; justify-content: space-around; align-items: flex-end;"> <div style="text-align: center;">  <p>7b Substrate</p> </div> <div style="text-align: center;">  <p>Epoxide</p> </div> </div> | | | |
|  <p>9b1</p> | | 1 : 2.3 | 59 % |
|  <p>9b2</p> | | 1 : 4 | 62 % |
|  <p>9b3</p> | | 1 : 2 | 48 % |
|  <p>9b4</p> | | - | 65 % |

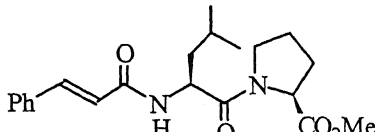
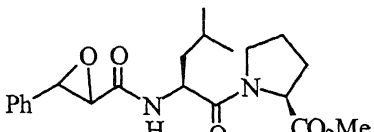
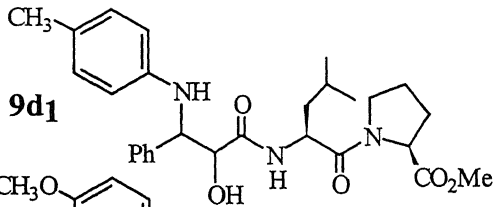
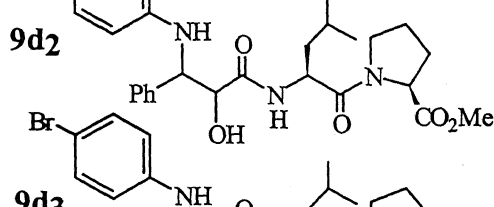
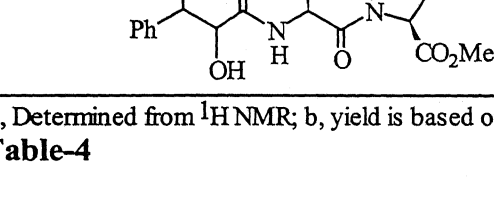
a, Determined from ¹H NMR; b, yield is based on the substrate

Table-3

Thus, N-cinnamoyl-dipeptides **7a-f** (Table-1) were subjected to the one-pot epoxidation and its opening by anilines **8** to get to a library of tripeptides **9**, containing β -phenylisoserine derivatives, incorporating the α -hydroxymethylenecarbonyl isostere.

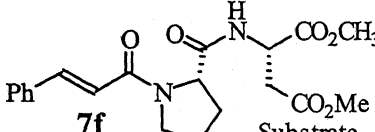
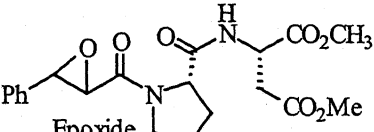
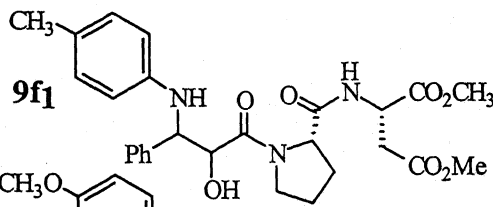
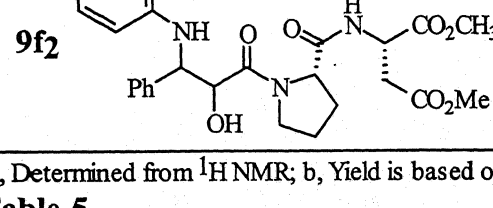
The opening of epoxides, in almost all cases, yielded a mixture of two diastereomeric products, of which one was predominant. The major diastereomer was assigned *anti* stereochemistry, based on the large coupling constant ($J \sim 6 - 7$ Hz), between methine

protons, compared with the corresponding *syn* diastereomers ($J \sim 4$ Hz).

| <div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;">  <p>7d Substrate</p> </div> <div style="text-align: center;">  <p>Epoxide</p> </div> </div> | | Product | Syn : Anti ^a | (Yield %) ^b |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--|------------------------------------------------------------------------------------------------------|-------------------------|------------------------|
| | |  <p>9d1</p> | 1 : 2.5 | 55 % |
| | |  <p>9d2</p> | 1 : 5 | 63 % |
| | |  <p>9d3</p> | 1 : 2 | 42 % |

a, Determined from ^1H NMR; b, yield is based on the substrate

Table-4

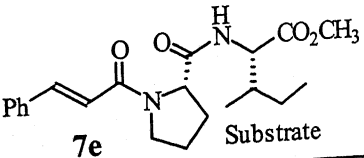
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|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--|-------------------------------------------------------------------------------------------------------|-------------------------|------------------------|
| | |  <p>9f1</p> | 1 : 3 | 56 % |
| | |  <p>9f2</p> | 1 : 4 | 60 % |

a, Determined from ^1H NMR; b, Yield is based on substrate

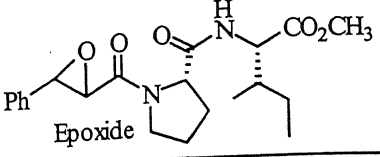
Table-5

The *anti* stereochemistry of the predominant diastereomers was also substantiated by chemical transformation. Compound **9** was subjected to Mitsunobu reaction conditions in the presence of diethylazodicarboxylate. The predominant *anti* diastereomer selectively

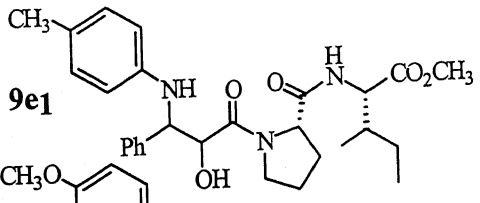
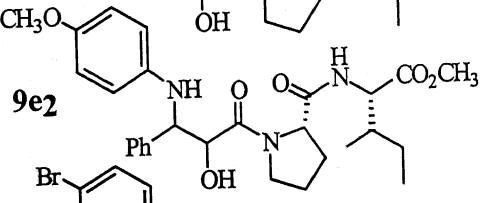
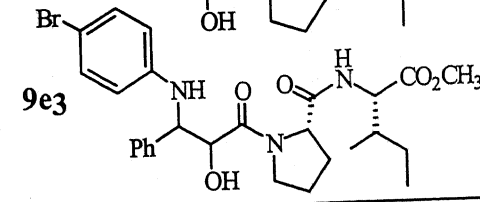
reacted, to yield the corresponding aziridine **10** in good yield (Scheme-6). The minor *syn*



7e
Substrate

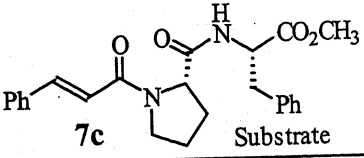


Epoxide

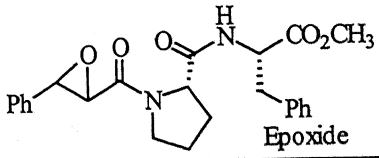
| Product | Syn : Anti ^a | (Yield %) ^b |
|-----------------------------------------------------------------------------------------------------|-------------------------|------------------------|
|  <p>9e1</p> | 1 : 3 | 48 % |
|  <p>9e2</p> | 1 : 5 | 54 % |
|  <p>9e3</p> | 1 : 3 | 45 % |

a, Determined from ¹H NMR; b, Yield is based on substrate

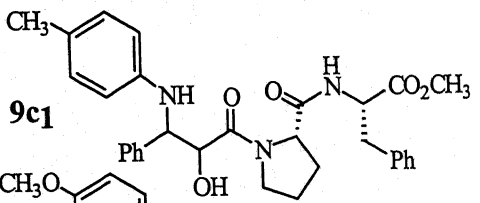
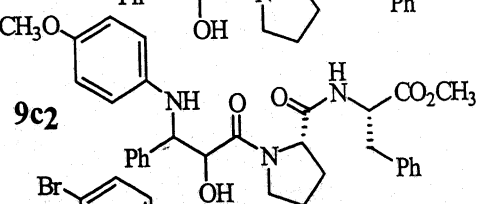
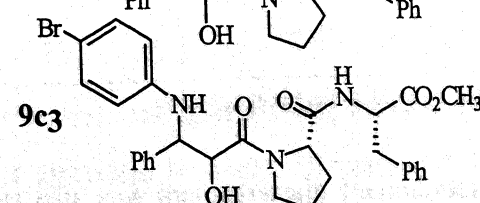
Table-6



7c
Substrate



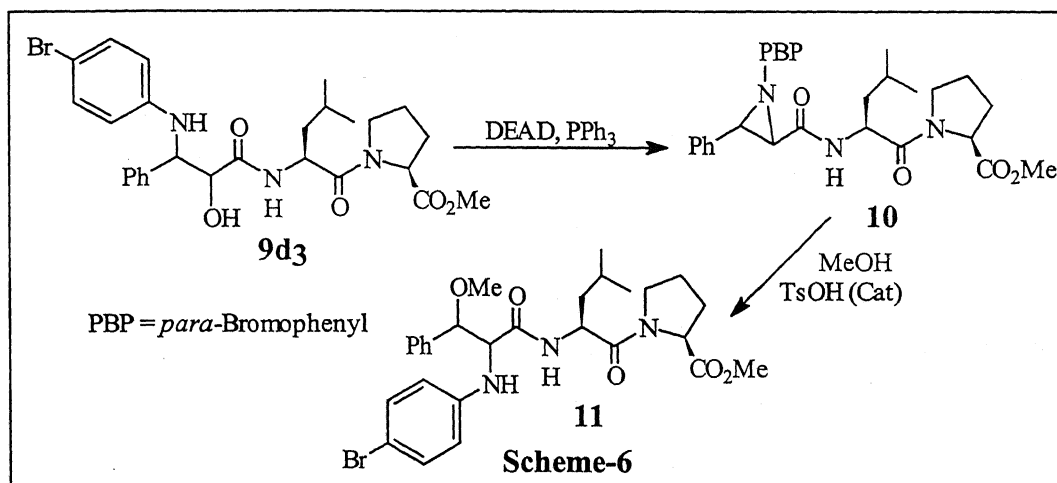
Epoxide

| Product | Syn : Anti ^a | (Yield %) ^b |
|-------------------------------------------------------------------------------------------------------|-------------------------|------------------------|
|  <p>9c1</p> | 1 : 2 | 42 % |
|  <p>9c2</p> | 1 : 4.5 | 56 % |
|  <p>9c3</p> | 1 : 2.5 | 50 % |

a, Determined from ¹H NMR; b, Yield is based on substrate

Table-7

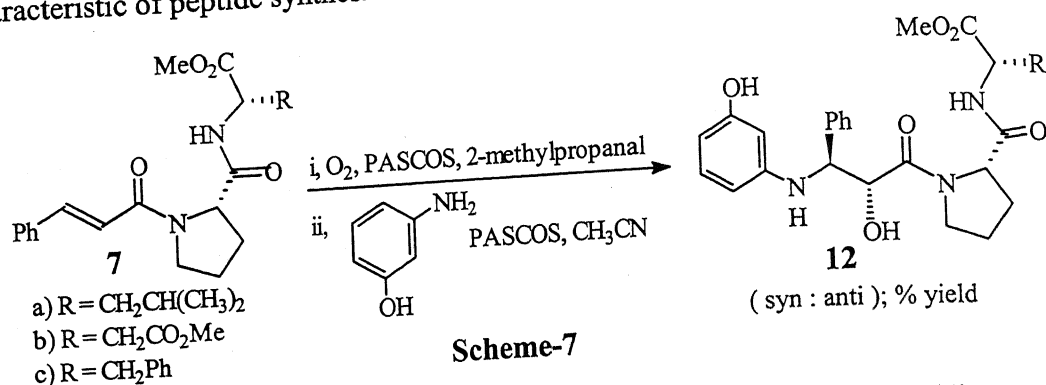
diastereomer was recovered during purification in column chromatography. Acid catalysed ring opening of the aziridine **10** with methanol, and purification of the reaction mixture, resulted in **11** in good yields. Thus, the β -phenylisoserine containing tripeptides can also be converted to the corresponding α -arylamino- β -substituted-phenylalanine derived pseudotripeptides, by following the aziridination and its subsequent opening, protocol.



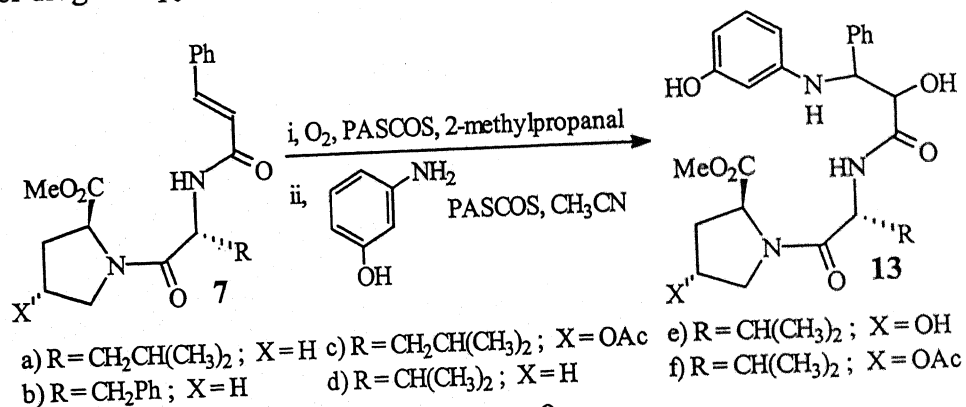
Thus, we have efficiently exploited the use of N-cinamoyl peptides **7** as versatile synthons in the synthesis of diverse libraries of tripeptides, containing the unnatural amino acid 3-amino-2-hydroxy-3-phenylpropionic acid (AHPPRA) named β -phenylisoserines (Figure-1), with a hydroxymethylcarbonyl (HMC) isostere adjacent to the scissile Phe-Pro amide bond, as probable lead structures for the inhibition of HIV PR.²⁶⁶ Based on the transition state mimetic concept we propose that these β -phenylisoserine derived peptides could be good transition state analogues of the known statine amino acids, discussed earlier. The potent statine acid derivatives, discussed earlier need several chemical transformations (some of them, in quite rigorous conditions) to be synthesized. This seemingly minor problem turns out to be otherwise in the prospective of need for time and cost effective methodologies to synthesize them for universal availability. We have introduced a simple, cost-effective and highly efficient methodology for the synthesis of tripeptides in any general permutation of amino acids, containing these β -phenylisoserine derivatives in good yields, by a one-pot synthetic protocol in the presence of PASCOS catalyst.

By this methodology we have discovered the N-cinnamoyl group as both an efficient N-terminal protecting group and a functional olefin exploited as versatile synthon in

hydroxyamination reactions for the synthesis of β-phenylisoserine-L-proline containing tripeptides in parallel. The advantage in the cinnamoyl group as an N-protecting group can be realized from the fact that a score of protection and de-protection steps, which are characteristic of peptide synthesis have been circumvented.



The synthesis of conformationally constrained small molecule peptides (discussed in detail in the next section) is a novel concept, for increasing the bioavailability and non-degradability of inhibitors of proteases. Such conformational constraints in peptides might lead to a conformation of the peptide, that mimics a turn motif like the β-turn. That the β-turn is one of the most common recognition elements, present at the protease active site, supplements vindication for the need of such conformationally constrained peptides as leads for drug therapy.



One of the most common methodologies used for the synthesis of cyclic peptides, is macrolactonisation. Various novel reagents exist for the macrolactonisation of appropriate precursors. We envisioned that opening of epoxides with a bi-functional aniline, which contains a handle for further lactonisation with the free carboxy ester in the N-cinnamoyl dipeptides, would be a well-sorted-after methodology for the synthesis of tripeptide precursors of choice, for cyclisation.

Added to this, such a protocol would generate tripeptides containing the unnatural amino acid statine analogue β -phenylisoserine, incorporating the α -hydroxycarbonyl isostere and hence, making these small peptides as excellent drug candidates for therapy of AIDS. That the N-cinnamoyl protecting group can be exploited to get to one of the most potent of isosteres, the hydroxymethylcarbonyl (HMC), which has shown picomolar inhibitory potencies when incorporated in ligands, cannot be over-emphasized.

Thus, in an effort to develop an efficient methodology for the synthesis of acyclic precursors that can be cyclised by macrolactonisation, we subjected the *in situ* generated epoxides of the CPX and CXP systems, *viz-a-viz* methyl-N-cinnamoyl-leu-prolinate, methyl-acetyl-N-cinnamoyl-leu-hydroxyprolinate, methyl-N-cinnamoyl-val-prolinate, methyl-acetyl-N-cinnamoyl-val-hydroxyprolinate, methyl-N-cinnamoyl-val-hydroxyprolinate, methyl-N-cinnamoyl-pro-leucinate, methyl-N-cinnamoyl-pro-aspartate, methyl-N-cinnamoyl-pro-phenylalaninate and methyl-N-(3-chlorocinnamoyl)-phe-prolinate, to opening by *meta*-amino phenol. After workup of the reaction mixture, we found that the opening took place regio- and chemo-selectively to yield the β -phenylisoserine containing tripeptide, in each of these cases. The yields of the opening reactions were found to be higher, when the reaction was performed in *one-pot*, from the N-cinnamoyl-dipeptide to the β -phenylisoserine containing tripeptide.

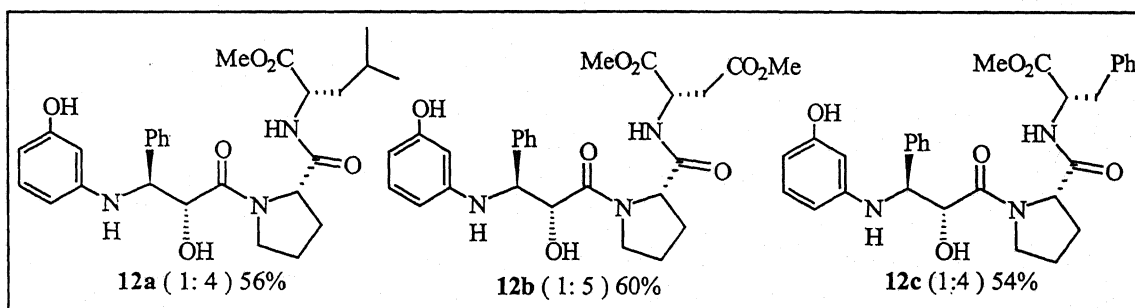
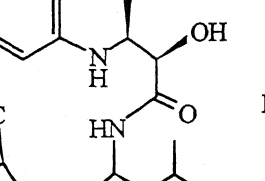
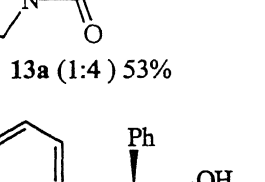


Table-8

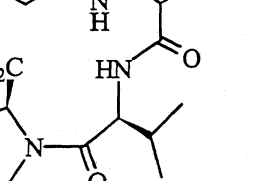
Thus, we describe a chemoselective opening of the *in situ* generated epoxide of N-cinnamoyl-dipeptides with *meta*-amino phenol leading to the synthesis of β -phenylisoserine and L-proline containing tripeptide isosteres, in the presence of polyaniline supported cobalt (II) salen (PASCOS) catalyst, as precursors for macrolactonisation. Our approach involves one-pot conversion of N-cinnamoyl peptides 1 or 4 to the corresponding tripeptides 3 or 6 respectively, by first forming the epoxide and subsequently its *in situ* opening with *meta* aminophenol in the presence of polyaniline supported cobalt (II) salen catalyst.



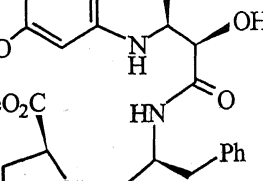
13a (1:4) 53%



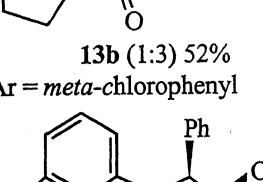
13b (1:3) 52%
Ar = meta-chlorophenyl



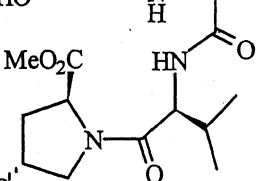
13c (1:4) 55%



13d (1:4) 47%



13e (1:2.5) 28%



13f (1:5) 50%

These tripeptides were isolated by circumventing the use of column chromatography via a non-aqueous work-up procedure. After completion of the reaction, the residue was dissolved in minimum volume of ethylacetate and carbontetrachloride (1 : 3) and to it hexane was added slowly, which resulted in the precipitation of the tripeptide in good yields. The mother liquor was concentrated and subjected to similar washing. After about three washes, the mother liquor mainly consisted of unreacted epoxide and amine, along with ~ 5 % of the tripeptide. The crude filtrates were washed once again, with CCl₄ and warm hexane and dried under vacuum, which gave the tripeptides in high purity (HPLC), most of them as solids.

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product (^1H NMR). The tripeptides were subjected to HPLC analysis and in each case, the major diastereomer was present in the ratio (3/4:1). The major diastereomer was assigned *anti* stereochemistry, based on the large coupling constant ($J \sim 6\text{--}7\text{Hz}$), between methine protons, compared with the corresponding *syn* diastereomers ($J \sim 4\text{ Hz}$). The absolute stereochemistry of the major *anti* diastereomer was assigned as (3R, 2R) for N-cinnamoyl-L-proline derived tripeptides, based on the absolute stereochemistry of the precursor epoxides which has been ascertained as (3S, 2R). It is interesting to know that no product arising out of the opening of the epoxide with phenolic group of *meta*-amino phenol was observed.

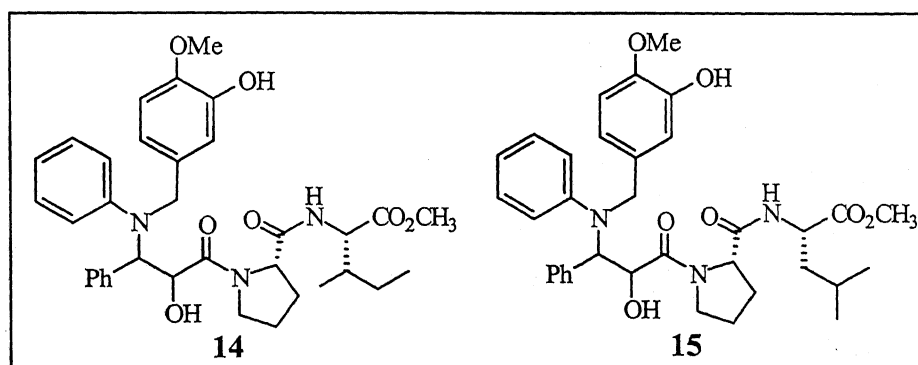
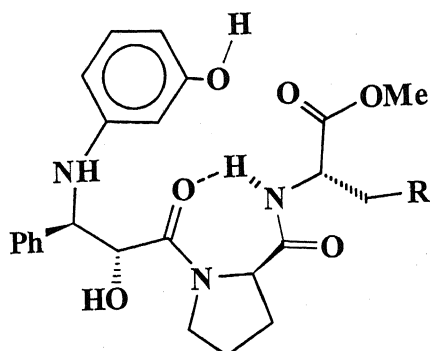


Table-10

Thus a tandem protocol involving the epoxidation of **7a-c** under aerobic conditions in the presence of PASCOS catalyst followed by the reaction with *meta*-aminophenol afforded the corresponding β -phenylisoserine derivatived tripeptides **12a-c** in one pot in good yields. Similarly, the N-cinnamoyl-dipeptides **7a-f** were transformed to the corresponding tripeptide **13a-f** respectively in good yields. These tripeptides were also isolated by non-aqueous work up using the precipitation techniques as described above for the isolation of **12**. Hither too, the *trans* diastereomer was found to be the major product for tripeptides **13a-f**. One-pot opening of *in situ* generated epoxides of N-cinnamoyl-dipeptides could also be achieved chemo-selectively by secondary amines containing phenolic groups, as exemplified by the synthesis of **14** and **15** (Table-10), from their respective CPX peptides, in the presence of PASCOS catalyst.

The presence of phenolic- and ester- groups in these tripeptides, make them as attractive precursors to β -turn mimic, mainly because an intramolecular-macrolactonisation of these peptides would result into the corresponding cyclic peptide which may behave as cyclic mimic of this turn (Figure-3). In the case of tripeptides derived from CPX peptide systems, presence of a proline might provide a γ -turn in the peptide system, which can

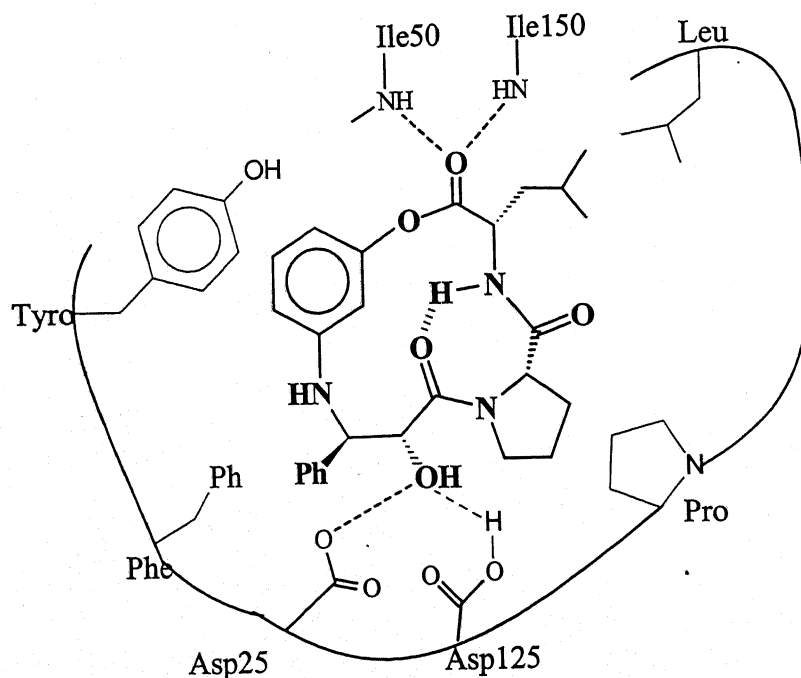
pre-organise the carbonyl and phenolic groups for easy macrolactonisation (Figure-2). We have synthesized tripeptides derived from β -phenylisoserine, with the hope that such



Proposed γ -turn Induced Pre-Organisation
For Facile Macrolactonisation of Precursor

Figure-2

peptides would mimic a β -turn conformation on cyclisation and hence become potent HIV protease inhibitors, incorporating one of the important recognition elements for binding at the protease active site. *Cyclisation of such tripeptides with an aromatic ring spacer may lock the beta turn conformation thereby rendering the resulting cyclic peptide more effective for binding with the HIV protease.*



γ -Turn Induced Cyclized, β -Turn Mimics, Containing
 β -Phenylisoserine Derivative

Figure-3

In conclusion, we have developed an efficient one-pot, PASCOS catalysed, regio- and chemo-selective transformation of N-cinnamoyl-dipeptides to the corresponding β -

phenylisoserine derived tripeptides, which are structural analogues of HIV PR inhibitors. Presented above, is also an efficient route for the access to proline derived core structures as potent HIV protease inhibitors. The chemo-selective opening of epoxides with *meta*-amino phenol provides a useful route to the synthesis of precursors to the cyclic peptides, which may be mimics of beta turn motifs. The efficiency and the easy non-aqueous work up procedure, make this a potentially useful methodology, for the synthesis of any tripeptide precursor of our choice, for cyclisation through macrolactonization.

Experimental Section

Materials and Methods

Acetonitrile, ethyl acetate, hexane, THF and all other solvents were purified by standard procedures. CoCl_2 was purchased from LOBA Indian Limited and dried at 140°C for 4 h before use. All the amino acids were bought from SpectroChem India Limited and used as such. Cinnamic acid, cinnamic acid derivative, ethylene diamine, primary aromatic amines, triethyl amine, acetic anhydride, methylchloroformate, 2-methylpropanal (isobutyraldehyde) were all procured commercially and were purified before use. The secondary amines have been prepared by the reductive amination of the corresponding aldehyde as per the literature procedure.¹⁸¹ The aldehydes were distilled before use. Amines were re-crystallized before use. Polyaniline supported Co(Salen) was prepared according to procedure developed in our lab.¹⁸² Column chromatography was performed on ACME silica-gel eluant. TLC was performed on ACME silica-gel-G coated glass plates and were irradiated using UV lamp. ^1H NMR spectra were recorded using Jeol PMX-60 system, Bruker WP-80, Jeol 300 FTNMR or JNMLA400 FTNMR machines in $\text{CCl}_4/\text{CDCl}_3$. Chemical shift are given relative to TMS in ppm(δ). Multiplicity is indicated using the following abbreviations: s(singlet), bs(broad singlet), d(doublet), dd(doublet of a doublet), ddd(doublet of a doublet of a doublet), dt(doublet of a triplet), td(triplet of a doublet), q(quartet) and m(multiplet). The FAB mass spectra were recorded on JEOL SX 102/DA 6000 mass spectrometer data system using Argon (6 Kv, 10 mA) as the FAB gas. Optical rotations were measured in Autopol® II/ Autopol® III polarimeters. All the known compounds were characterized by comparing with the literature data. IR spectra were recorded on Perkin Elmer 683 spectrophotometer, using either a neat sample or a solution in $\text{CCl}_4/\text{CH}_2\text{Cl}_2$ and solids were examined as KBr pellets and the values are reported in ν_{max} (cm^{-1}). HPLC analyses were done with Rainin System fitted with Dynamax® SD-200 pump and detected with Groton PDA solonet Diode Array Detector.

Preparation of Polyaniline

Freshly distilled aniline 10 mL (109.5 mmole) was dissolved in 125 mL of 1.5 M HCl, and a solution of ammoniumpersulfate (54.8 mmole) in 1.5 M HCl (125 mL) was added to it at 0°C . Since aniline polymerization is strongly exothermic, the oxidant must be added slowly over a period of 1h. After the addition of the oxidant, the reaction was stirred further for 4 h. The polyaniline hydrochloride precipitate was separated by filtration and washed consecutively with water (3 X 30 mL), methanol (2 X 25 mL), and diethyl ether (2 X 15 mL) to remove the oligomers and any of the reaction side products. The polymer was then vacuum-dried until constant mass. Deprotonation of polyaniline hydrochloride was achieved with aqueous ammonia (3 wt%). Deprotonated polymer was again washed with water, methanol, and diethyl ether and dried until constant mass (~3 gm). Polyaniline is quite stable to air and can be stored indefinitely in closed glass vials.

Preparation of Polyaniline supported cobalt (II) salen (PASCOS)

Cobaltous salen (200 mg) and polyaniline (200 mg) were added to a solution of acetic acid (25 mL) in acetonitrile (25 mL) and stirred at ambient temperature for 36 h. The resultant catalyst was filtered off and washed first with acetic acid (3 X 10 mL) and then thoroughly with acetonitrile until the filtrate was colorless. The resulting residue was dried in an air oven at 100°C for 2 h to afford the black (or blackish brown) colored

catalyst. Polyaniline supported cobalt (II) salen is stable to atmosphere and can be stored indefinitely in closed vials.

General Procedure for the Synthesis of methyl-L-amino ester hydrochloride

To an ice cold, stirring suspension of the L-amino acid (1 equivalent) in methanol (1 mL/mmol) was added thionylchloride (1.1 equivalents) drop wise from a dropping funnel. The clear solution that resulted was stirred at room temperature for 3 h. Methanol was removed in vacuo and the resulting solid was washed with dry ether. Drying under vacuo yielded the amino ester hydrochloride in nearly quantitative yields, which was used for further reaction in the next step without any further purification.

In general it was observed that coupling reactions with methyl-amino ester hydrochlorides were better yielding when these were freshly synthesised before use for subsequent synthesis, due to the hygroscopic nature of most of these salts.

Synthesis of methyl-L-prolinate hydrochloride

To an ice cold, stirring suspension of L-proline (5.75 gm, 50 mmol) in methanol (50 mL) was added thionylchloride (4 mL, 55 mmol) drop wise from a dropping funnel. The clear solution that resulted was stirred at room temperature for 3 h. Methanol was removed in vacuo and the resulting gummy liquid was washed with dry ether. Drying under vacuo yielded methyl-L-proline hydrochloride as a gum in nearly quantitative yields, which was used for further reaction in the next step without any further purification.

Synthesis of methyl-L-leucinate hydrochloride

To an ice cold, stirring suspension of L-leucine (3.93 gm, 30 mmol) in methanol (30 mL) was added thionylchloride (2.42 mL, 33 mmol) drop wise from a dropping funnel. The clear solution that resulted was stirred at room temperature for 3 h. Methanol was removed in vacuo and the resulting solid was washed with dry ether. Drying under vacuo yielded methyl-L-leucine hydrochloride in nearly quantitative yields as a crystalline white solid, which was used for further reaction in the next step without any further purification.

Synthesis of methyl-L-valinate hydrochloride

To an ice cold, stirring suspension of L-valine (2.54 gm, 20 mmol) in methanol (20 mL) was added thionylchloride (1.61 mL, 22 mmol) drop wise from a dropping funnel. The clear solution that resulted was stirred at room temperature for 3 h. Methanol was removed in vacuo and the resulting solid was washed with dry ether. Drying under vacuo yielded methyl-L-valinate hydrochloride in nearly quantitative yields as a crystalline white solid, which was used for further reaction in the next step without any further purification.

Synthesis of methyl-L-isoleucinate hydrochloride

To an ice cold, stirring suspension of L-isoleucine (1.96 gm, 15 mmol) in methanol (15 mL) was added thionylchloride (1.21 mL, 16.5 mmol) drop wise from a dropping funnel. The clear solution that resulted was stirred at room temperature for 3 h. Methanol was removed in vacuo and the resulting solid was washed with dry ether. Drying under vacuo yielded methyl-L-isoleucine hydrochloride in nearly quantitative yields as a crystalline white solid, which was used for further reaction in the next step without any further purification. It is advisable in the case of reactions with methyl-L-isoleucinate to freshly synthesize it every time before use.

Synthesis of methyl-L-phenylalaninate hydrochloride

To an ice cold, stirring suspension of L-phenylalanine (3.3 gm, 20 mmol) in methanol (20 mL) was added thionylchloride (1.61 mL, 22 mmol) drop wise from a dropping funnel. The clear solution that resulted was stirred at room temperature for 3 h. Methanol was removed in vacuo and the resulting solid was washed with dry ether. Drying under vacuo yielded methyl-L-phenylalaninate hydrochloride in nearly quantitative yields as a crystalline white solid, which was used for further reaction in the next step without any further purification.

Synthesis of methyl-L-aspartate hydrochloride

To an ice cold, stirring suspension of L-aspartic acid (2.66 gm, 20 mmol) in methanol (20 mL) was added thionylchloride (3.22 mL, 44 mmol) drop wise from a dropping funnel. The clear solution that resulted was stirred at room temperature for 3 h. Methanol was removed in vacuo and the resulting solid was washed with dry ether. Drying under vacuo yielded dimethyl-L-aspartate hydrochloride in nearly quantitative yields as a crystalline white solid, which was used for further reaction in the next step without any further purification.

Synthesis of methyl-L-tyrosinate hydrochloride

To an ice cold, stirring suspension of L-tyrosine (1.81 gm, 10 mmol) in methanol (10 mL) was added thionylchloride (0.8 mL, 11 mmol) drop wise from a dropping funnel. The clear solution that resulted was stirred at room temperature for 3 h. Methanol was removed in vacuo and the resulting solid was washed with dry ether. Drying under vacuo yielded methyl-L-tyrosinate hydrochloride in nearly quantitative yields as a crystalline white solid, which was used for further reaction in the next step without any further purification.

Synthesis of methyl-L-serinate hydrochloride

To an ice cold, stirring suspension of L-serine (1.05 gm, 10 mmol) in methanol (10 mL) was added thionylchloride (0.8 mL, 11 mmol) drop wise from a dropping funnel. The clear solution that resulted was stirred at room temperature for 3 h. Methanol was removed in vacuo and the resulting solid was washed with dry ether. Drying under vacuo yielded methyl-L-serinate hydrochloride in nearly quantitative yields as a crystalline white solid, which was used for further reaction in the next step without any further purification.

Synthesis of methyl-*trans*-4-hydroxy-L-prolinate hydrochloride

To an ice cold, stirring suspension of 4-*trans*-hydroxy-L-proline (1.31 gm, 10 mmol) in methanol (10 mL) was added thionylchloride (0.8 mL, 11 mmol) drop wise from a dropping funnel. The clear solution that resulted was stirred at room temperature for 3 h. Methanol was removed in vacuo and the resulting solid was washed with dry ether. Drying under vacuo yielded methyl-*trans*-4-hydroxy-L-prolinate hydrochloride in nearly quantitative yields as a crystalline white solid, which was used for further reaction in the next step without any further purification.

General Procedure for the Synthesis of methyl-N-cinnamoyl-amino ester

Method A

To a stirring, ice cold solution of cinnamic acid (1 equivalent) and triethylamine (1 equivalent) in THF (1.5 mL/mmol) was added methylchloroformate (1 equivalent) and the mixture was stirred vigorously for 2 minutes.* After which, a solution of the amino ester hydrochloride (1.1 equivalents) in DMSO (0.5 mL/mmol) was added followed by triethylamine (2.2 equivalents) dissolved in THF (1 mL/mmol). The reaction vessel was allowed to warm to room temperature and vigorously stirred for further 3-4 h. Triethylamine hydrochloride was filtered off on a sintered funnel under suction and washed with THF. Removal of solvent from the filtrate under vacuo yielded a residue which was dissolved in EtOAc (~ 2mL/mmol) and washed with saturated aqueous solution of NaHCO₃, water and brine. Drying (Na₂SO₄) and evaporation of solvent under vacuo yielded the crude product which was further purified by column chromatography (EtOAc:Hexane) to yield the required product usually as good solids in good yields.

* On prolonging beyond this time, usually methyl cinnamate is formed in healthy quantities as a side product.

Method B

To a stirring ice cold solution of cinnamoyl chloride (1 equivalent) in dichloromethane (1 mL/mmol) was added the amino ester hydrochloride (1.1 equivalents) followed by a solution of triethylamine (2.2 equivalents) in dichloromethane (1 mL/mmol) drop wise through a dropping funnel. After complete addition of triethylamine, the reaction mixture was vigorously stirred for a further 5-6 h and then diluted with dichloromethane (1 mL/mmol). Work up as described in method A, with saturated aqueous solution of NaHCO₃, water and brine and purification by column chromatography yielded the N-cinnamoyl amino ester in good yields.

In general, it was observed that yields of methyl-N-cinnamoyl-amino esters were better by method A, than by method B.

Synthesis of methyl-N-cinnamoyl-L-leucinate 7a

To a stirring, ice cold solution of cinnamic acid (1.48 gm, 10mmol) and triethylamine (1.4 mL, 10mmol) in THF (15 mL) was added methylchloroformate (0.77 mL, 10 mmol) and the mixture was stirred vigorously for 2 minutes. After which, a solution of methyl leucinate hydrochloride (2 gm, 10 mmol) in DMSO (4-5 mL) was added followed by triethylamine (3.1 mL, 22 mmol) dissolved in THF (15 mL). The reaction vessel was allowed to warm to room temperature and vigorously stirred for further 3-4 h. Triethylamine hydrochloride was filtered off on a sintered funnel under suction and washed with THF. Removal of solvent from the filtrate under vacuo yielded a residue which was stirred with saturated aqueous solution of NaHCO₃ (20 mL) for 15 minutes. The bicarbonate layer was decanted and the resulting semi solid washed with water (2X10 mL). A yellow solid precipitated, which was filtered off on a suction funnel and dried in a dessicator under vacuum, on a cellulose filter paper for 10 h. Purification of the resulting solid by column chromatography (EtOAc:Hexane = 1:5.25) (TLC - R_f = 0.5; hexane:ethylacetate 5:1) yielded the above said compound as a crystalline white solid (M.P.= 128 °C) in good yields (85%). $[\alpha]_D^{25} = +20^\circ$ (c = 0.01, CH₂Cl₂).

^1H NMR, 60 MHz, CDCl_3 , 7.73(d, $J = 8.2\text{Hz}$, 1H), 7.60(d, $J = 16\text{Hz}$, 1H), 7.35(s, 3H), 6.70(d, $J = 16\text{Hz}$, 1H), 4.90(dd, $J = 10.2\text{Hz}$ & $J = 5.6\text{Hz}$, 1H), 3.82(s, 3H), 1.73(dd, $J = 8\text{Hz}$ & $J = 1.6\text{Hz}$, 2H), 1.21-1.18(m, 1H), 0.96(d, $J = 6.5\text{Hz}$, 6H).

Synthesis of methyl-N-cinnamoyl-L-valinate 7b

To a stirring, ice cold solution of cinnamic acid (1.48 gm, 10 mmol) and triethylamine (1.4 mL, 10 mmol) in THF (15 mL) was added methylchloroformate (0.77 mL, 10 mmol) and the mixture was stirred vigorously for 2 minutes. After which, a solution of methyl valinate hydrochloride (1.84 gm, 11 mmol) in DMSO (4-5 mL) was added followed by triethylamine (3.1 mL, 22 mmol) dissolved in THF (15 mL). The reaction vessel was allowed to warm to room temperature and vigorously stirred for further 3-4 h. Triethylamine hydrochloride was filtered off on a sintered funnel under suction and washed with THF. Removal of solvent from the filtrate under vacuo yielded a residue which was taken in EtOAc (25 mL) and the organic layer was washed with saturated aqueous solution of NaHCO_3 (2X10 mL), water (2X10mL) and brine (1X10mL). Drying (anhydrous Na_2SO_4) and evaporation of solvent yielded a residue which was purified by column chromatography (EtOAc:Hexane = 1:5.25) (TLC - $R_f = 0.45$; hexane:ethylacetate 5:1) yielded the above said compound as a gum in good yields (68%).

^1H NMR, 60 MHz, CDCl_3 , δ 7.63(D, $J = 16\text{Hz}$, 1H), 7.33 (s, 5H), 6.90 (d, $J = 9\text{Hz}$, 1H), 6.57 (d, $J = 16\text{Hz}$, 1H), 4.80 (dd, $J = 8.5\text{Hz}$ & $J = 5.5\text{Hz}$, 1H), 3.80(s, 3H), 2.30 (ds, $J = 6\text{Hz}$ & $J = 2\text{Hz}$, 1H), 1.22 (d, $J = 6.5\text{Hz}$, 6H)

Synthesis of methyl-N-cinnamoyl-L-phenylalaninate 20c

To a stirring ice cold solution of cinnamoyl chloride (1.65 gm, 10 mmol) in dichloromethane (15 mL) was added methylphenylalaninate hydrochloride (2.37 gm, 11 mmol) followed by a solution of triethylamine (3.1 mL, 22 mmol) in dichloromethane (15 mL) drop wise through a dropping funnel. After complete addition of triethylamine, the reaction mixture was vigorously stirred for a further 5-6 h and then diluted with dichloromethane (15 mL). The organic layer was washed with saturated aqueous solution of NaHCO_3 , water and brine. Drying (Na_2SO_4) and evaporation of solvent under vacuo yielded the crude product which was further purified by column chromatography (EtOAc:Hexane = 1:5) (TLC - $R_f = 0.5$; hexane ethylacetate 4:1) to yield the required product as a crystalline white solid (M.P. = 78°C) in good yields (80 %).

^1H NMR 60 MHz, CDCl_3 , δ 7.68 (d, $J = 15.8\text{ Hz}$, 1H), 7.62 - 7.50 (m, 5H), 7.45 - 7.39 (m, 5H), 5.10 (dd, $J = 13.5\text{ Hz}$ & 9 Hz , 1H), 3.69 (s, 3H), 3.15 (d, $J = 6\text{ Hz}$, 2H)

Synthesis of methyl-N-cinnamoyl-L-prolinate 22

To a stirring, ice cold solution of cinnamic acid (1.15 gm, 10 mmol) and triethylamine (1.4mL, 10 mmol) in THF (15 mL) was added methylchloroformate (0.77 mL, 10 mmol) and the mixture was stirred vigorously for 2 minutes. After which, a solution of the methyl prolinate hydrochloride (1.82 gm, 11 mmol) in DMSO (4-5mL) was added followed by triethylamine (3.1 mL, 22 mmol) dissolved in THF (15 mL). The reaction vessel was allowed to warm to room temperature and vigorously stirred for further 3-4 h. Triethylamine hydrochloride was filtered off on a sintered funnel under suction and washed with THF. Removal of solvent from the filtrate under vacuo yielded a residue, which was stirred with a saturated aqueous solution of NaHCO_3 (20mL) for 15 minutes. The bicarbonate layer was decanted and the resulting semi solid was washed with water (10 mL). An yellow solid precipitated, which was filtered off on a suction funnel and

dried in a dessicator under vacuum, on a cellulose filter paper for 8-10 h. Purification of the resulting solid by column chromatography (EtOAc:Hexane = 1:2.45) (TLC - R_f = 0.35; hexane:ethylacetate 3.3:1) yielded the above said compound as a crystalline solid (M.P.= 58 °C) in good yields (74 %).

^1H NMR, CDCl_3 , 60 MHz, δ , 7.55 (d, J = 12 Hz, 1H), 7.20 (m, 5H), 6.6 (d, J = 12 Hz, 1H), 4.5 (m, 1H), 3.6 (bs, 1H), 2.0 (m, 4H)

Synthesis of methyl-N-(3'-chloro-cinnamoyl)-L-phenylalaninate 7c

To a stirring, ice cold solution of 3'-chloro-cinnamic acid (1.83 gm, 10 mmol) and triethylamine (1.4 mL) in THF (1.5 mL) was added methylchloroformate (0.77 mL, 10 mmol) and the mixture was stirred vigorously for 2 minutes. After which, a solution of the methyl phenylalaninate hydrochloride (2.37 gm, 11 mmol) in DMSO (4-5 mL) was added followed by triethylamine (3.1 mL, 22 mmol) dissolved in THF (15 mL). The reaction vessel was allowed to warm to room temperature and vigorously stirred for further 4-5 h. Triethylamine hydrochloride was filtered off on a sintered funnel under suction and washed with THF. Removal of solvent from the filtrate under vacuo yielded a residue which was taken in EtOAc (30 mL) and washed with saturated aqueous solution of NaHCO_3 (2X10 mL), water (2X10mL) and brine (1X10mL). Drying (anhydrous Na_2SO_4) and evaporation of solvent yielded a residue which was purified by column chromatography (EtOAc:Hexane = 1:3) (TLC - R_f = 0.40; hexane:ethylacetate 3:1) yielded the above said compound as a solid (M.P.= 78 °C) in good yields (72 %).

^1H NMR, 60 MHz, CDCl_3 , δ 7.62 (d, J = 16Hz, 1H), 7.38 - 7.15 (m, 9H), 6.53 (d, J = 16Hz, 1H), 5.08 (dd, J = 13.5Hz & J = 6Hz, 1H), 3.85 (s, 3H), 3.22 (d, J = 8.2Hz, 2H)

General Procedure for the Synthesis of N-cinnamoyl-amino acid

Method A

To an ice cold solution of cinnamoylchloride (1 equivalent) in dichloromethane (1 mL/mmol) was added the L-amino acid (1 equivalent). To the stirring mixture a solution of triethylamine (2.2 equivalents) in dichloromethane (1 mL/mmol) was added drop wise at such a rate that the vessel temperature did not exceed 10 °C. After complete addition, the reaction mixture was warmed to room temperature and stirring was continued for 4-5 h. The solvent was evaporated in vacuo. To the resulting residue was added while stirring, aqueous solution of 1N HCl until no more acidification occurred (formation of milky white precipitate ceased). The aqueous suspension of yellowish white solid was filtered on a sintered funnel under suction and dried by spreading on cellulose filter paper for 10 h in a dessicator, to yield the N-cinnamoyl amino acid in excellent yields.

Method B

To a solution of the methyl-N-cinnamoyl amino ester (1 equivalent) in MeOH (4 mL/mmol) was added a solution of $\text{LiOH}\cdot\text{H}_2\text{O}$ (1.2-1.5 equivalents) in water (1 mL/mmol)* and stirred at room temperature until completion of reaction (TLC - complete disappearance of starting material). Methanol was removed under vacuo and the aqueous part was acidified with an aqueous solution of 1N HCl by drop wise addition and simultaneous vigorous stirring. After complete acidification (no more milky white precipitate occurs on addition of dilute HCl), the resulting residue - if it was a solid- was filtered off on a sintered funnel under suction and dried on cellulose filter paper for 10 - 12 h in a dessicator; or -if it was a gum- was extracted with dichloromethane, dried

(anhydrous sodium sulphate) and concentrated under vacuo; to yield the N-cinnamoyl amino acid, usually in good yields as a solid or gum.

*It might be required to use warm water to dissolve $\text{LiOH}\cdot\text{H}_2\text{O}$ in case of larger scale reactions (> 20 mmol). In which case, after dissolving in warm water, it is better to cool the solution before addition to the methanolic solution of the methyl ester.

It is notable that the TLC patterns of most of these N-cinnamoyl peptides with free carboxyl group appear as white streaks, on visualising in iodine chamber.

Synthesis of N-cinnamoyl-L-proline 3

To an ice cold solution of cinnamoylchloride (1.67 gm, 10 mmol) in dichloromethane (15 mL) was added L-proline (1.15 gm, 10 mmol). To the stirring mixture a solution of triethylamine (3.1 mL, 22 mmol) in dichloromethane (20 mL) was added drop wise at such a rate that the vessel temperature did not exceed 10°C . After complete addition, the reaction mixture was warmed to room temperature and stirring was continued for 4-5 h. The solvent was evaporated in vacuo. To the resulting residue was added while stirring, aqueous solution of 1N HCl until no more acidification occurred (formation of milky white precipitate ceased). The aqueous suspension of yellowish white solid was filtered on a sintered funnel under suction and dried by spreading on cellulose filter paper for 10 h in a dessicator, to yield N-cinnamoyl proline as a white solid (M.P. = 162°C) in excellent yields (96 %).

Synthesis of N-cinnamoyl-L-leucine 8a

To a solution of the methyl-N-cinnamoyl leucinate (1.38 gm, 5 mmol) in MeOH (20 mL) was added a solution of $\text{LiOH}\cdot\text{H}_2\text{O}$ (205 mg, 5 mmol) in water (5 mL) and stirred at room temperature until completion of reaction (TLC - EtOAc:hexane - 1:3 - complete disappearance of starting material). Methanol was removed under vacuo and the aqueous part was acidified with an aqueous solution of 1N HCl by drop wise addition and simultaneous vigorous stirring. After complete acidification (no more milky white precipitate occurs on addition of dilute HCl), the resulting residue was extracted with dichloromethane (30mL), dried (anhydrous sodium sulphate) and concentrated under vacuo to yield N-cinnamoyl-L-leucine, in good yields (85 %) as a hygroscopic gum.

Synthesis of N-cinnamoyl-L-Valine 8b

To a solution of the methyl-N-cinnamoyl valinate (2.09 g gm, 8 mmol) in MeOH (16 mL) was added a solution of $\text{LiOH}\cdot\text{H}_2\text{O}$ (330 mg, 8 mmol) in water (2 mL) and stirred at room temperature until completion of reaction (TLC - EtOAc:hexane - 1:3 - complete disappearance of starting material). Methanol was removed under vacuo and the aqueous part was acidified with an aqueous solution of 1N HCl by drop wise addition and simultaneous vigorous stirring. After complete acidification (no more milky white precipitate occurs on addition of dilute HCl), the resulting residue was extracted with dichloromethane (3X10mL), dried (anhydrous sodium sulphate) and concentrated under vacuo to yield N-cinnamoyl-L-valine, in good yields (72 %) as a gum.

Synthesis of N-(3'-chloro-cinnamoyl)-L-phenylalanine 8c

To a solution of the methyl-N-(3'-chloro-cinnamoyl)-L-phenylalaninate (1.72 gm, 5 mmol) in MeOH (20 mL) was added a solution of $\text{LiOH}\cdot\text{H}_2\text{O}$ (205 mg, 5 mmol) in water (4 mL) and stirred at room temperature until completion of reaction (TLC - EtOAc:hexane - 1:1.5 - complete disappearance of starting material). Methanol was removed under vacuo and the aqueous part was acidified with an aqueous solution of 1N HCl by drop wise addition and simultaneous vigorous stirring. After complete

acidification (no more milky white precipitate occurs on addition of dilute HCl), the resulting residue was extracted with dichloromethane (3X10mL), dried (anhydrous sodium sulphate) and concentrated under vacuo to yield N-(3'-chloro-cinnamoyl)-L-phenylalanine, in good yields (78 %) as a hygroscopic solid.

Synthesis of N-cinnamoyl-phenylalanine (8d)

To a stirring ice cold solution of triethylamine (3.1 mL, 22 mmol) in acetonitrile (30 mL) was added L-phenylalanine (1.65 gm, 10 mmol). A solution of cinnamoyl chloride (1.67 gm, 10 mmol) in acetonitrile (15 mL) was added drop wise to this solution at such a rate that the reaction vessel temperature did not exceed 10 °C. After complete addition, the reaction mixture was warmed to room temperature and stirring was continued for 4-5 h. Following acidification and isolation as described in method A yielded N-cinnamoyl-L-phenylalanine as a sticky white solid in good yields (85 %).

General Procedure for the Synthesis of methyl-N-cinnamoyl-proline-Xaa - CPX systems (Xaa = linear methyl- α -amino ester)

A stirring solution of N-cinnamoyl-proline (1 equivalent) and triethylamine (1 equivalent) in THF (1.5 mL/mmol) was cooled to -5 - -10 °C* in an ice-salt bath and to it was added methylchloroformate (1 equivalent) and stirred vigorously for 50 - 60 seconds.* Then a solution of the methyl-aminoester hydrochloride (HCl.Xaa.OMe) (1.1 equivalents) in DMSO (0.5 mL/mmol) was added followed by a solution of triethylamine (2.2 equivalents) in THF (1.5 mL/mmol). The mixture was warmed to room temperature by removal of the ice-salt bath and vigorously stirred for further 4 h. Triethylamine hydrochloride was filtered off on a sintered funnel under suction. Removal of solvent from the filtrate under vacuo yielded a residue, which was dissolved in EtOAc (~2mL/mmol) and washed with saturated aqueous solution of NaHCO₃, water and brine. Drying (Na₂SO₄) and evaporation of solvent under vacuo yielded the crude product which was further purified by column chromatography (EtOAc:Hexane) to yield the required product usually as good solids in moderate to good yields in high purity. Most of these solids can be recrystallised in EtOAc-hexane to give good crystalline compounds.

*Aberrations from this temperature and time usually resulted in poor yields and recovery of the acid, or in the formation of methyl-N-cinnamoyl-L-prolinate as side product.

General Procedure for the Synthesis of methyl-N-cinnamoyl-Xaa-prolinate - CXP systems (Xaa = linear methyl α -amino ester)

A stirring solution of N-cinnamoyl-L-amino acid (1 equivalent) and triethylamine (1 equivalent) in THF (1-1.5 mL/mmol) was cooled to -5 - -10 °C* in an ice-salt bath and to it was added methylchloroformate (1 equivalent) and stirred vigorously for 50 - 60 seconds.* Then a solution of methyl proline hydrochloride / methyl (4-*trans*-hydroxy) proline hydrochloride (1.1 equivalents) in DMSO (0.5 mL/mmol) was added followed by a solution of triethylamine (2.2 equivalents) in THF (1 mL/mmol). The mixture was warmed to room temperature by removal of the ice bath and vigorously stirred for further 4 h. Triethylamine hydrochloride was filtered off on a sintered funnel under suction. Removal of solvent from the filtrate under vacuo yielded a residue, which was dissolved in EtOAc (~2mL/mmol) and washed with saturated aqueous solution of NaHCO₃, water and brine. Drying (Na₂SO₄) and evaporation of solvent under vacuo yielded the crude product which was further purified by column chromatography (EtOAc:Hexane) to yield the required product usually as semi solids in moderate to good yields.

*Aberrations from this temperature and time usually resulted in poor yields and recovery of the acid, or in the formation of the corresponding methyl-N-cinnamoyl-L-amino esters as side product.

Synthesis of methyl-N-cinnamoyl-L-proline-L-leucinate (5a)

A stirring solution of N-cinnamoyl-proline (2.45 gm, 10 mmol) and triethylamine (1.4 mL, 10 mmol) in THF (15 mL) was cooled to -10°C in an ice-salt bath and to it was added methylchloroformate (0.77 mL, 10 mmol) and stirred vigorously for 50 - 60 seconds. Then a solution of methyl-L-leucinate hydrochloride (2.00 gm, 11 mmol) in DMSO (0.5 mL) was added followed by a solution of triethylamine (3.1 mL, 22 mmol) in THF (15 mL). The mixture was warmed to room temperature by removal of the ice bath and vigorously stirred for further 4 h. Triethylamine hydrochloride was filtered off on a sintered funnel under suction and washed twice with THF. Removal of solvent from the filtrate under vacuo yielded a residue, which was dissolved in EtOAc (30 mL) and washed with saturated aqueous solution of NaHCO_3 (2X10mL), water (2X10mL) and brine (1X10mL). Drying (Na_2SO_4) and evaporation of solvent under vacuo yielded the crude product which was further purified by column chromatography (EtOAc:Hexane-1:1.5) (TLC - $R_f = 0.40$; hexane:ethylacetate 1.5:1) to yield methyl-N-cinnamoyl-L-proline-L-leucinate as a solid (M.P. = 110°C) in good yield (80 %); $[\alpha]_D^{25} = -170.5$ ($c = 0.01$, CH_2Cl_2).

^1H NMR (400 MHz, CDCl_3) δ 7.74 (d, $J = 15.3$ Hz, 1H), 7.66 (d, $J = 7.32$ Hz, 1H), 7.56 - 7.54 (m 2H), 7.39 - 7.35 (m, 3 H), 6.76 (d, $J = 15.6$ Hz, 1H), 4.57 - 4.47 (m, 2H), 3.73 (s, 3H), 3.70 - 3.61 (m, 2H), 2.51 - 2.46 (m, 1H), 2.06 - 2.03 (m, 1H), 2.21 - 2.12 (m, 1H), 1.89 - 1.81 (m, 2H), 1.67 - 1.56 (m, 2H), 0.91 (d, $J = 5.6$ Hz, 3H), 0.88 (d, $J = 5.6$ Hz, 3H); IR (KBr) ν_{max} 3400, 3030, 2950, 2880, 1730, 1640, 1600, 1480, 1440 cm^{-1} ; FT IR (CH_2Cl_2) : 3278, 3059, 2956.5, 2872.7, 1744.8, 1649.6, 1598.1, 1542.1, 1498.0, 1425.3

Synthesis of methyl-N-cinnamoyl-L-proline-L-isoleucinate 5b

A stirring solution of N-cinnamoyl-proline (1.23 gm, 5 mmol) and triethylamine (0.7 mL, 5 mmol) in THF (10 mL) was cooled to -5°C in an ice-salt bath and to it was added methylchloroformate (0.39 mL, 5 mmol) and stirred vigorously for 50 - 60 seconds. Then a solution of methyl-L-isoleucinate hydrochloride (998 mg, 5.5 mmol) in DMSO (4-5 mL) was added followed by a solution of triethylamine (1.6 mL, 11 mmol) in THF (10 mL). The mixture was warmed to room temperature by removal of the ice bath and vigorously stirred for further 4 h. Triethylamine hydrochloride was filtered off on a sintered funnel under suction. Removal of solvent from the filtrate under vacuo yielded a residue, which was dissolved in EtOAc (20 mL) and washed with saturated aqueous solution of NaHCO_3 (2X10mL), water (2X10mL) and brine (1X10mL). Drying (Na_2SO_4) and evaporation of solvent under vacuo yielded the crude product which was further purified by column chromatography (EtOAc:Hexane-1:1.5) (TLC - $R_f = 0.45$; hexane:ethylacetate 1.5:1) to yield methyl-N-cinnamoyl-L-proline-L-isoleucinate as a solid (M.P. = $151-154^{\circ}\text{C}$) in good yields (70 %).

^1H NMR, 60 MHz, CDCl_3 , δ 7.92 (d, $J = 8$ Hz, 1H), 7.76 (d, $J = 15$ Hz, 1H), 7.65 - 7.24 (m, 5H), 6.92(d, $J = 15$ Hz, 1H), 4.87(d, $J = 8.0$ Hz, 1H), 4.56 (dd, $J = 12$ Hz & 8 Hz, 1H), 3.84 (s, 3H), 3.80 - 3.62(m, 2H), 2.72 - 2.61 (m, 1H), 2.53 - 2.41 (m, 1H), 2.24 - 1.96 (m, 3H), 1.32 (dt, $J = 22.4$ Hz & 11.2 Hz, 1H), 0.94 (dd, $J = 9.6$ Hz & 3 Hz, 6H).

Synthesis of dimethyl-N-cinnamoyl-L-proline-L-aspartate 5c

A stirring solution of N-cinnamoyl-proline (2.45 gm, 10 mmol) and triethylamine (1.4 mL, 10 mmol) in THF (15 mL) was cooled to -5 °C in an ice-salt bath and to it was added isobutylchloroformate (1.30 mL, 10 mmol) and stirred vigorously for 50 - 60 seconds. Then a solution of dimethyl-L-aspartate hydrochloride (2.18 gm, 11 mmol) in DMSO (4-5 mL) was added followed by a solution of triethylamine (3.1 mL, 22 mmol) in THF (15 mL). The mixture was warmed to room temperature by removal of the ice bath and vigorously stirred for further 4 h. Triethylamine hydrochloride was filtered off on a sintered funnel under suction. Removal of solvent from the filtrate under vacuo yielded a residue, which was dissolved in EtOAc (30 mL) and washed with saturated aqueous solution of NaHCO₃ (2X10mL), water (2X10mL) and brine (1X10mL). Drying (Na₂SO₄) and evaporation of solvent under vacuo yielded the crude product which was further purified by column chromatography (EtOAc:Hexane-1:1.8) (TLC - R_f = 0.40; hexane:ethylacetate 1.8:1) to yield dimethyl-N-cinnamoyl-L-proline-L-aspartate as a gum in moderate yields (65 %)

80 MHz ¹H NMR CDCl₃, δ 7.68 (d, J = 16 Hz, 1H), 7.48 (d, J = 7.6 Hz, 1H), 7.33(s, 5H), 6.68 (d, J = 16 Hz, 1H), 4.76 (d, J = 9 Hz, 1H), 4.66 (dd, J = 8.4 Hz & 3.6 Hz, 1H), 3.84 - 3.79 (m, 1H), 3.72 (s, 3H), 3.69 (s, 3H), 3.66 - 3.60 (m, 1H), 2.89 (dd, J = 8 Hz & 5.6 Hz, 1H), 2.10 - 1.97 (m, 2H).

Synthesis of methyl-N-cinnamoyl-L-proline-L-phenylalaninate (5d)

A stirring solution of N-cinnamoyl-proline (2.45 gm, 10 mmol) and triethylamine (1.4 mL, 10 mmol) in THF (15 mL) was cooled to -5 °C in an ice-salt bath and to it was added methylchloroformate (0.77 mL, 10 mmol) and stirred vigorously for 50 - 60 seconds. Then a solution of methyl-L-phenylalaninate hydrochloride (2.37 gm, 11 mmol) in DMSO (5 mL) was added followed by a solution of triethylamine (3.1 mL, 11 mmol) in THF (15 mL). The mixture was warmed to room temperature by removal of the ice bath and vigorously stirred for further 4 h. Triethylamine hydrochloride was filtered off on a sintered funnel under suction. Removal of solvent from the filtrate under vacuo yielded a residue, which was stirred with saturated aqueous solution of NaHCO₃ (20mL) for 15 minutes upon which, a solid precipitated. The aqueous bicarbonate layer was decanted and the solid was washed with water (2X10mL) and filtered on a sintered funnel under suction. Drying of the solid on a cellulose filter paper in a desiccator under vacuo yielded the crude product which was further purified by column chromatography (EtOAc:Hexane-1:1.5) (TLC - R_f = 0.40; hexane:ethylacetate 1.5:1) to yield methyl-N-cinnamoyl-L-proline-L-phenylalaninate as a solid (M.P. = 41-43 °C) in good yields (80 %). [α]_D²⁵ = -102° (c=0.004, CH₂Cl₂).

¹H NMR, CDCl₃, 400 MHz, δ 7.80 (d, J = 8 Hz, 1H), 7.73 (d, J = 15.4 Hz, 1H), 7.57 - 7.51 (m, 2H), 7.43 - 7.39 (m, 3H), 7.19 - 7.10 (m, 5H), 6.67 (d, J = 15.4 Hz, 1H), 4.84 (dt, J = 8.1 Hz & 5.4 Hz, 1H), 4.73 (d, J = 7.6 Hz, 1H), 3.73 (s, 3H), 3.54 - 3.51 (m, 2H), 3.19 (dd, J = 13.9 Hz & 5.4 Hz, 1H), 2.96 (dd, J = 13.9 Hz & 8 Hz, 1H), 2.42 - 2.39 (m, 1H), 1.98 - 1.900 (m, 2H), 1.79 - 1.72 (m, 1H).

Synthesis of methyl-N-cinnamoyl-L-leucine-prolinate (9a)

A stirring solution of N-cinnamoyl-L-leucine (1.30 gm, 5 mmol) and triethylamine (0.7 mL, 5 mmol) in THF (10 mL) was cooled to -10 °C in an ice-salt bath and to it was

added methylchloroformate (0.38 mL, 5 mmol) and stirred vigorously for 50 - 60 seconds. Then a solution of methyl-L-prolinate hydrochloride (916 mg, 5.5 mmol) in DMSO (2 mL) was added followed by a solution of triethylamine (1.6 mL, 11 mmol) in THF (10 mL). The mixture was warmed to room temperature by removal of the ice bath and vigorously stirred for further 4 h. Triethylamine hydrochloride was filtered off on a sintered funnel under suction. Removal of solvent from the filtrate under vacuo yielded a residue, which was dissolved in EtOAc (20 mL) and washed with saturated aqueous solution of NaHCO_3 (2X10 mL), water (2X10 mL) and brine (1X10 mL). Drying (Na_2SO_4) and evaporation of solvent under vacuo yielded the crude product which was further purified by column chromatography (EtOAc:Hexane-1:1.5) (TLC - R_f = 0.50; hexane:ethylacetate 1.5:1) to yield methyl-N-cinnamoyl-L-leucine-L-prolinate as a gum, in good yields (61 %).

^1H NMR, 60 MHz, CDCl_3 , δ 7.72(d, J = 16 Hz, 1H), 7.59(d, J = 8.9 Hz, 1H), 7.50-7.16(m, 5H), 6.47(d, J = 16 Hz, 1H), 5.16(dd, J = 9 Hz & J = 5.6 Hz, 1H), 4.58(dd, J = 10.2 Hz & J = 4.8 Hz, 1H), 4.13(d, J = 11.2 Hz, 1H), 3.65(s, 3H), 3.64-3.60(m, 1H), 2.30-2.12(m, 3H), 2.10-1.99(m, 1H), 1.76-1.49(m, 3H), 0.96(d, J = 5.6 Hz, 6H).

Synthesis of methyl-N-cinnamoyl-L-leucine-(4-*trans*-hydroxy)L-prolinate

A stirring solution of N-cinnamoyl-L-leucine (1.3 gm, 5 mmol) and triethylamine (0.7 mL, 5 mmol) in THF (10 mL) was cooled to -10°C in an ice-salt bath and to it was added methylchloroformate (0.38 mL, 5 mmol) and stirred vigorously for 50 - 60 seconds. Then a solution of methyl-4-*trans*-hydroxy-L-prolinate hydrochloride (1.00 gm, 5.5 mmol) in DMSO (3 mL) was added followed by a solution of triethylamine (1.6 mL, 11 mmol) in THF (15 mL). The mixture was warmed to room temperature by removal of the ice bath and vigorously stirred for further 4 h. Triethylamine hydrochloride was filtered off on a sintered funnel under suction. Removal of solvent from the filtrate under vacuo yielded a residue, which was dissolved in EtOAc (20 mL) and washed with saturated aqueous solution of NaHCO_3 (2X10 mL), water (2X10 mL) and brine (1X10 mL). Drying (Na_2SO_4) and evaporation of solvent under vacuo yielded the crude product which was further purified by column chromatography (EtOAc:Hexane-1.5:1) (TLC - R_f = 0.50; hexane : ethylacetate 1:1.5) to yield methyl-N-cinnamoyl-L-leucine-(4-*trans*-hydroxy)L-prolinate as a gum, in moderate yields (52 %).

^1H NMR, 80 MHz, CDCl_3 , δ 7.56 (d, J = 16.8 Hz, 1H), 7.42 - 7.06 (m, 5H), 6.88 (d, J = 11.2 Hz, 1H), 6.33 (d, J = 16.8 Hz, 1H), 4.78 (dd, J = 17.6 Hz & 9.6 Hz, 1H), 4.59 - 4.31 (m, 2H), 3.84 (d, J = 8.0 Hz, 1H), 3.67 (dd, J = 8.0 Hz & J = 4.8 Hz, 1H), 3.61(s, 3H), 2.08 (dd, J = 15.2 Hz & 5.6 Hz, 2H), 1.75 - 1.22 (m, 3H), 0.86 (d, J = 6.6 Hz, 6H).

Synthesis of methyl-N-cinnamoyl-L-valine-L-prolinate (9c)

A stirring solution of N-cinnamoyl-L-valine (1.24 gm, 5 mmol) and triethylamine (0.7 mL, 5 mmol) in THF (10 mL) was cooled to -10°C in an ice-salt bath and to it was added methylchloroformate (0.38 mL, 5 mmol) and stirred vigorously for 50 - 60 seconds. Then a solution of methyl-L-prolinate hydrochloride (916 mg, 5 mmol) in DMSO (2 mL) was added followed by a solution of triethylamine (1.6 mL, 11 mmol) in THF (10 mL). The mixture was warmed to room temperature by removal of the ice bath and vigorously stirred for further 4 h. Triethylamine hydrochloride was filtered off on a sintered funnel under suction. Removal of solvent from the filtrate under vacuo yielded a residue, which was dissolved in EtOAc (20 mL) and washed with saturated aqueous

solution of NaHCO_3 (2X10mL), water (2X10mL) and brine (1X10mL). Drying (Na_2SO_4) and evaporation of solvent under vacuo yielded the crude product which was further purified by column chromatography (EtOAc:Hexane-1:1.5) (TLC - R_f = 0.50; hexane : ethylacetate 2:1) to yield methyl-N-cinnamoyl-L-valine-L-prolinate as a gum, in moderate yields (50 %).

^1H NMR, 80 MHz, CDCl_3 , δ 7.59 (d, J = 17Hz, 1H), 7.25 (s, 5H), 6.75 (d, J = 8Hz, 1H), 6.53 (d, J = 17Hz, 1H), 4.68 (dd, J = 17.6Hz & 6.5Hz, 1H), 4.34 (dd, J = 14.4Hz & 2.9Hz, 1H), 3.62 (s, 3H), 3.50 - 3.22 (m, 2H), 2.15 - 1.98 (m, 2H), 1.95 - 1.58 (m, 3H), 0.89 (d, J = 8.8Hz, 6H)

Synthesis of methyl-N-cinnamoyl-L-valine-L-(4-*trans*-hydroxy)-L-prolinate (9e)

A stirring solution of N-cinnamoyl-L-valine (2.47 gm, 10 mmol) and triethylamine (1.4 mL, 10 mmol) in THF (25 mL) was cooled to -10°C in an ice-salt bath and to it was added methylchloroformate (0.77 mL, 10 mmol) and stirred vigorously for 50 - 60 seconds. Then a solution of methyl-4-*trans*-hydroxy-L-prolinate hydrochloride (2.00 gm, 11 mmol) in DMSO (5 mL) was added followed by a solution of triethylamine (3.1 mL, 22 mmol) in THF (20 mL). The mixture was warmed to room temperature by removal of the ice bath and vigorously stirred for further 4 h. Triethylamine hydrochloride was filtered off on a sintered funnel under suction. Removal of solvent from the filtrate under vacuo yielded a residue, which was dissolved in EtOAc (40 mL) and washed with saturated aqueous solution of NaHCO_3 (2X10 mL), water (2X10 mL) and brine (1X10 mL). Drying (Na_2SO_4) and evaporation of solvent under vacuo yielded the crude product which was further purified by column chromatography (EtOAc:Hexane-1.5:1) (TLC - R_f = 0.40; hexane : ethylacetate 1:1.5) to yield methyl-N-cinnamoyl-L-valine-(4-*trans*-hydroxy)-L-prolinate as a gum, in moderate yields (44 %).

^1H NMR, 80 MHz, CDCl_3 , δ 7.63 (d, J = 8.8Hz, 1H), 7.56 (d, J = 19Hz, 1H), 7.48 - 7.03 (m, 5H), 6.56 (d, J = 19Hz, 1H), 4.64 (dd, J = 18.4Hz & 9.6Hz, 1H), 4.55 (d, J = 8Hz, 1H), 4.51-4.42 (m, 1H), 4.12 (dd, J = 17.6Hz & 9Hz, 2H), 3.69 (s, 3H), 2.30 (dt, J = 19.2Hz & 9.6Hz, 1H), 2.28 - 2.16 (m, 1H), 0.98 (d, J = 8Hz, 6H)

Synthesis of methyl-N-(3'-chloro-cinnamoyl)-L-phenylalanine-L-prolinate (9f)

A stirring solution of N-(3'-chloro-cinnamoyl)-L-phenylalanine (1.00 gm, 3 mmol) and triethylamine (0.42 mL, 3 mmol) in THF (10 mL) was cooled to -10°C in an ice-salt bath and to it was added methylchloroformate (0.23 mL, 3 mmol) and stirred vigorously for 50 - 60 seconds. Then a solution of methyl L-phenylalaninate hydrochloride (711 mg, 3.3 mmol) in DMSO (1.5 mL) was added followed by a solution of triethylamine (0.92 mL, 6.6 mmol) in THF (10 mL). The mixture was warmed to room temperature by removal of the ice bath and vigorously stirred for further 4 h. Triethylamine hydrochloride was filtered off on a sintered funnel under suction. Removal of solvent from the filtrate under vacuo yielded a residue, which was dissolved in EtOAc (30 mL) and washed with saturated aqueous solution of NaHCO_3 (2X10 mL), water (2X10 mL) and brine (1X10 mL). Drying (Na_2SO_4) and evaporation of solvent under vacuo yielded the crude product which was further purified by column chromatography (EtOAc:Hexane-1:1.5) (TLC - R_f = 0.50; hexane : ethylacetate 1:1) to yield methyl-N-(3'-chloro-cinnamoyl)-L-phenylalanine-L-prolinate as a solid (M.P. = $76 - 78^\circ\text{C}$), in moderate yields (58 %).

^1H NMR, 60 MHz, CDCl_3 , δ 7.62 (d, J = 6 Hz, 1H), 7.46 (d, J = 16.5 Hz, 1H), 7.50 - 7.35 (m, 3H), 7.33 (s, 5H), 7.18 (d, J = 8Hz, 1H), 7.00 (d, J = 3Hz, 1H), 6.46 (d, J = 16.5Hz, 1H), 5.15 (dd, J = 15Hz & 8Hz, 1H), 4.45 (dd, J = 13.5 Hz & 6Hz, 1H), 4.00 (d, J = 8.8Hz, 1H), 3.68 (s, 3H), 3.57 (d, J = 8Hz, 1H), 3.12 (d, J = 8.5Hz, 2H), 2.25 - 2.13 (m, 1H), 2.18 (bs, 1H), 2.00 (bs, 1H), 1.96 (m, 1H)

Synthesis of N-cinnamoyl-L-phenylalanine-L-serinate (12)

A stirring solution of N-cinnamoyl-L-phenylalanine (1.47 gm, 5 mmol) and triethylamine (0.7 mL, 5 mmol) in THF (15 mL) was cooled to -10°C in an ice-salt bath and to it was added methylchloroformate (0.38 mL, 5 mmol) and stirred vigorously for 50 - 60 seconds. Then a solution of methyl L-serinate hydrochloride (861 mg, 5.5 mmol) in DMSO (3 mL) was added followed by a solution of triethylamine (1.6 mL, 11 mmol) in THF (1.5 mL). The mixture was warmed to room temperature by removal of the ice bath and vigorously stirred for further 10 h. Triethylamine hydrochloride was filtered off on a sintered funnel under suction. Removal of solvent from the filtrate under vacuo yielded a residue, which was dissolved in EtOAc (40 mL) and washed with saturated aqueous solution of NaHCO_3 (2X10 mL), water (2X10 mL) and brine (1X10 mL). Drying (Na_2SO_4) and evaporation of solvent under vacuo yielded the crude product which was further purified by column chromatography (EtOAc:Hexane-1:1.5) (TLC - R_f = 0.50; hexane : ethylacetate 1:1) to yield methyl-N-cinnamoyl-L-phenylalanine-L-serinate as a gum, in moderate yields (48 %).

^1H NMR, 60 MHz, CDCl_3 , δ 7.65 (d, J = 17 Hz, 1H), 7.55 (d, J = 8 Hz, 1H), 7.35 (s, 5H), 7.12 (s, 5H), 6.35 (d, J = 17 Hz, 1H), 4.98 (dd, J = 14 Hz & 7.5 Hz, 1H), 4.55 (dt, J = 8 Hz & 4.5 Hz, 1H), 3.86 (d, J = 5.6 Hz, 2H), 3.66 (s, 3H), 3.15 (d, J = 9Hz, 2H)

Synthesis of methyl-N-cinnamoyl-L-valine-L-tyrosinate (13)

A stirring solution of N-cinnamoyl-L-valine (1.23 gm, 5 mmol) and triethylamine (0.7 mL, 5 mmol) in THF (10 mL) was cooled to -10°C in an ice-salt bath and to it was added methylchloroformate (0.38 mL, 5 mmol) and stirred vigorously for 50 - 60 seconds. Then a solution of methyl L-tyrosinate hydrochloride (1.27 gm, 5.5 mmol) in DMSO (5 mL) was added followed by a solution of triethylamine (1.6 mL, 11 mmol) in THF (15 mL). The mixture was warmed to room temperature by removal of the ice bath and vigorously stirred over night. Triethylamine hydrochloride was filtered off on a sintered funnel under suction. Removal of solvent from the filtrate under vacuo yielded a residue, which was dissolved in CH_2Cl_2 (40 mL) and washed with saturated aqueous solution of NaHCO_3 (2X10 mL), water (2X10mL) and brine (1X10mL). Drying (Na_2SO_4) and evaporation of solvent under vacuo yielded the crude product which was further purified by column chromatography (EtOAc:Hexane-1:1) (TLC - R_f = 0.3; hexane : ethylacetate 1:1) to yield methyl-N-cinnamoyl-L-valine-L-tyrosinate as a gum, in moderate yields (32 %).

^1H NMR, 60 MHz, CDCl_3 , δ 7.62-7.43 (m, 5H), 7.12 (d, J = 8Hz, 1H), 6.96 (d, J = 4Hz, 2H), 6.75 (d, J = 4Hz, 2H), 4.88 (dd, J = 19.4Hz & 8.5Hz, 1H), 4.66 (d, J = 9Hz, 1H), 3.75 (s, 3H), 3.03 (d, J = 8Hz, 2H), 2.00 (bs, 1H), 0.97 (d, J = 6.5Hz, 6H)

Synthesis of methyl-N-cinnamoyl-L-valine-(4-*trans*-acetoxy)-L-prolinate (9d)

Methyl-N-cinnamoyl-L-valine-(4-*trans*-hydroxy)-L-prolinate (1.07 gm, 3 mmol) was dissolved in dry DCM (15 mL) and the reaction vessel was cooled in an ice bath.

Triethylamine (0.33 mL, 3.3 mmol) and DMAP (catalytic amount) were added to it followed by freshly distilled acetic anhydride (454 mg). The reaction mixture was stirred at ice-cold temperature for 0.5 h and then stirred at room temperature over night. The reaction was quenched with water (2 mL) and stirred for 15 minutes. The reaction mixture was subsequently washed with water (2X10mL), saturated aqueous solution of NaHCO₃ (2X10mL) and brine (2X10mL). Drying the organic layer (anhyd. Na₂SO₄) and concentration in vacuo resulted in a residue which was purified by column chromatography (EtOAc:Hexane-1:1) (TLC - R_f = 0.5; hexane : ethylacetate 2:3) to yield methyl-N-cinnamoyl-L-valine-(4-*trans*-acetoxy)-L-prolinate as a gum, in good yields (78 %).

¹H NMR, 80 MHz, CDCl₃, δ 7.53 (d, J = 19.2 Hz, 1H), 7.38 - 7.16 (m, 5H), 6.69(d, J = 8.8 Hz, 1H), 6.38 (d, J = 19.2 Hz, 1H), 5.27 (dt, J = 8.8Hz & 4Hz, 1H), 4.53 (dd, J = 12.8Hz & 5.6Hz, 1H), 4.48 (dd, J = 10.4Hz & 4Hz, 1H), 3.86 (dd, J = 11.2Hz & 5.6Hz, 1H), 3.73 (dd, J = 5.8Hz & 3.2Hz, 1H), 3.53 (s, 3H), 2.31 (dt, J = 8Hz & J = 3.8Hz, 1H), 2.12 (dd, J = 11.2Hz & 3.7Hz, 1H), 1.94 (s, 3H), 1.91-1.79 (m, 1H), 0.89 (d, J = 8Hz, 6H).

Synthesis of methyl-N-cinnamoyl-L-leucine-(4-*trans*-acetoxy)-L-prolinate (9b)

Methyl-N-cinnamoyl-L-leucine-(4-*trans*-hydroxy)-L-prolinate (930 mg, 2.5 mmol) was dissolved in dry DCM (15 mL) and the reaction vessel was cooled in an ice bath. Triethylamine (0.38 mL, 2.7 mmol) and DMAP (catalytic amount) were added to it followed by freshly distilled acetic anhydride (380 mg). The reaction mixture was stirred at ice-cold temperature for 0.5 h and then stirred at room temperature over night. The reaction was quenched with water (2 mL) and stirred for 15 minutes. The reaction mixture was subsequently washed with water (2X10mL), saturated aqueous solution of NaHCO₃ (2X10mL) and brine (1X10mL). Drying the organic layer (anhyd. Na₂SO₄) and concentration in vacuo resulted in a residue which was purified by column chromatography (EtOAc:Hexane-1:1) (TLC - R_f = 0.55; hexane : ethylacetate 2:3) to yield methyl-N-cinnamoyl-L-leucine-(4-*trans*-acetoxy)-L-prolinate as a gum, in good yields (82 %).

¹H NMR, 60 MHz, CDCl₃, δ 7.85 (d, J = 8Hz, 1H), 7.70 (d, J = 16Hz, 1H), 7.62 - 7.35 (m, 5H), 6.65 (d, J = 16Hz, 1H), 5.50 (m, 1H), 5.05 (dd, J = 12Hz & 5.6Hz, 1H), 4.82 (dd, J = 9Hz & 4Hz, 1H), 4.30 (dd, J = 9Hz & 4.8Hz, 1H), 4.10 (d, J = 10.2Hz, 1H), 3.85 (s, 3H), 2.43 (dd, J = 8Hz & 2.4Hz, 2H), 2.23 (s, 3H), 1.87 - 1.60 (m, 3H), 0.97 (dd, J = 8Hz & 4.5Hz, 6H)

Synthesis of methyl-N-cinnamoyl-(O-acetyl)-L-tyrosinate (14)

Methyl-N-cinnamoyl-L-tyrosinate (687 mg, 2 mmol) was dissolved in dry DCM (12 mL) and the reaction vessel was cooled in an ice bath. Triethylamine (0.31 mL, 2.2 mmol) and DMAP (catalytic amount) were added to it followed by freshly distilled acetic anhydride (0.303 mg, 3 mmol). The reaction mixture was stirred at ice-cold temperature for 0.5 h and then stirred at room temperature over night. The reaction was quenching with water (1 mL) and stirred for 15 minutes. The reaction mixture was subsequently washed with water (2X10mL), saturated aqueous solution of NaHCO₃ (2X10mL) and brine (1X10mL). Drying the organic layer (anhyd. Na₂SO₄) and concentration in vacuo resulted in a residue which was purified by column chromatography (EtOAc:Hexane-1:2) (TLC -

$R_f = 0.4$; hexane : ethylacetate 1.5:1) to yield methyl-N-cinnamoyl-(O-acetyl)-L-tyrosinate as a gum, in good yield (76 %).

^1H NMR, 400 MHz, CDCl_3 , δ 7.72 (d, $J = 16\text{Hz}$, 1H), 7.63-7.15 (m, 5H), 7.50 (d, $J = 5.6\text{Hz}$, 2H), 7.09 (d, $J = 5.6\text{Hz}$, 2H), 6.50 (d, $J = 16\text{Hz}$, 1H), 5.05 (dd, $J = 16.8\text{Hz}$ & 7.4Hz, 1H), 3.72 (s, 3H), 3.17 (d, $J = 7.2\text{Hz}$, 2H), 2.23 (s, 3H)

Synthesis of methyl-N-cinnamoyl-(O-crotonoyl)-L-tyrosinate (15)

Methyl-N-cinnamoyl-L-tyrosinate (343 mg, 1 mmol) was dissolved in dry DCM (6 mL) and the reaction vessel was cooled in an ice bath. Triethylamine (0.16 mL, 1.1 mmol) and DMAP (catalytic amount) were added to it followed by freshly distilled acetic anhydride (0.15 mg, 1.5 mmol). The reaction mixture was stirred at ice-cold temperature for 0.5 h and then stirred at room temperature over night. The reaction was quenching with water (0.5 mL) and stirred for 15 minutes. The reaction mixture was subsequently diluted with DCM (10 mL) and washed with water (2X5 mL), saturated aqueous solution of NaHCO_3 (2X5 mL) and brine (1X5 mL). Drying the organic layer (anhydrous Na_2SO_4) and concentration in vacuo resulted in a residue, which was purified by column chromatography (EtOAc in Hexane-33 %) (TLC - $R_f = 0.4$; hexane : ethylacetate 2.5:1) to yield methyl-N-cinnamoyl-(O-crotonoyl)-L-tyrosinate as a gum, in good yields (65 %).

^1H NMR, 400 MHz, CDCl_3 , δ 7.85 (d, $J = 16.1\text{ Hz}$, 1H), 7.65 & 7.62 (d, $J = 3.9$, 1H), 7.49 - 7.47 (m, 1H), 7.41 - 7.40 (m, 1H), 7.35 - 7.33 (m, 3H), 7.17 (d, $J = 8.2\text{ Hz}$, 2H), 7.10 (d, $J = 8.2\text{ Hz}$, 2H), 7.03 (d, $J = 8.1\text{ Hz}$, 1H), 6.60 (d, $J = 16.1\text{ Hz}$, 1H), 6.44 (dd, $J = 15.6\text{ Hz}$ & 5.6 Hz, 1H), 5.03 (td, $J = 13.7\text{ Hz}$ & 6 Hz, 1H), 3.73 (s, 3H), 3.19 (dd, $J = 14.6\text{ Hz}$ & 8 Hz, 2H), 1.95 (dd, $J = 7.2\text{ Hz}$ & 1.4 Hz, 3H)

General Procedure for the Polyaniline Supported Cobalt(II)salen (PASCOS) catalysed Epoxidation of N-Cinnamoyl-dipeptides:

To a solution of the N-cinnamoyl-dipeptide methyl ester (1 equivalent) in CH_3CN (5 mL/mmol) was added 2-methylpropanal (2 equivalents) and PASCOS catalyst[#] (~5 mg) and the contents were stirred under oxygen atmosphere at RT for 12 h. After this time, a fresh lot of the catalyst and 2-methylpropanal (2 equivalents)* were added to the reaction mixture and allowed to stir, until complete conversion of the olefin (TLC).[@] The catalyst was filtered off on a cintered funnel and acetonitrile was removed in vacuo. The resulting residue was taken in EtOAc and washed successively with saturated solution of NaHCO_3 , water and brine. Separating the organic phase, drying and concentration in vacuo, yielded the corresponding oxirane containing peptide in high purity and yields (HPLC). These epoxides were further subjected to column chromatography (silica gel; EtOAc:Hexane) for purification to get to the pure epoxides in excellent yields.

[#] The catalyst should be washed thoroughly with acetonitrile until the wash is constantly colorless. Impurities in the catalyst were found to hinder the formation of epoxide.

* The aldehyde needs to be added in four equivalents for optimum and best conversions. Isobutyraldehyde can also be taken in lots of 3 equivalents and then 1 equivalent. However, lesser reaction times were realised by adding it in lots of 2 equivalents each.

[@] Pre-coated fluorescent silica plates and Silica gel-G coated glass plates were used as immobile phase. TLC plates were visualised in I2 chamber and under U.V. light, (254 nm). Characteristically, all the N-cinnamoyl peptides show up as excellent bright spots under U.V. light, whereas the epoxides show up as very light spots under U.V. light.

Synthesis of methyl-N-(3-phenylglycidyl)-proline-leucinate (10a)

To a solution of the methyl-N-cinnamoyl-proline-leucinate (744 mg, 2 mmol) in CH_3CN (10 mL) was added 2-methylpropanal (288 mg, 4 mmol) and PASCOS catalyst (~5 mg) and the contents were stirred under oxygen atmosphere at RT for 12 h. After this time, a fresh lot of the catalyst and 2-methylpropanal (288 mg, 4 mmol) were added to the reaction mixture and allowed to stir, until complete conversion of the olefin (TLC - R_f = 0.5; EtOAc:Hexane - 1:1). The catalyst was filtered off on a cintered funnel and acetonitrile was removed in vacuo. The resulting residue was taken in EtOAc (25 mL) and washed successively with saturated solution of NaHCO_3 (2X10 mL), water (2X10 mL) and brine (1X10 mL). Separating the organic phase, drying (Na_2SO_4) and concentration in vacuo, yielded the corresponding oxirane containing peptide **10a** in high purity and yields (HPLC). This was further subjected to column chromatography (silica gel; EtOAc:Hexane - 2:3) for purification to get to the pure epoxide **10a** as solid (M.P. = $^\circ\text{C}$) in excellent yields (86 %) $[\alpha]_D^{25} = -124^\circ$ ($c = 0.01$, CH_2Cl_2).

^1H NMR 400 MHz, CDCl_3 , δ 7.34 - 7.20 (m, 5 H), 7.15 (d, $J = 7.9$ Hz, 1H), 4.67 (d, $J = 5.6$ Hz, 1H), 4.49 (t, $J = 4.2$ Hz, 1H), 4.03 (d, $J = 3.6$ Hz, 1H), 3.73 (s, 3H), 3.61 (d, $J = 3.6$ Hz, 1H), 3.57 (m, 2H), 2.39 - 1.89 (m, 1H), 0.93 (dd, $J = 6.6$ Hz, 6H); MS m/z 389, 339, 307, 281, 269, 244, 216, 209, 181, 154, 136; IR ν_{max} 3200 (br), 3030, 2910, 2880, 1760, 1655 cm^{-1}

Synthesis of methyl-N-(3-phenylglycidyl)-proline- phenylalaninate (10b)

To a solution of the methyl-N-cinnamoyl-proline-phenylalaninate (609 mg, 1.5 mmol) in CH_3CN (7.5 mL) was added 2-methylpropanal (216 mg, 3 mmol) and PASCOS catalyst (~5 mg) and the contents were stirred under oxygen atmosphere at RT for 12 h. After this time, a fresh lot of the catalyst and 2-methylpropanal (216 mg, 3 mmol) were added to the reaction mixture and allowed to stir, until complete conversion of the olefin (TLC - R_f = 0.5; EtOAc:Hexane - 1:1). The catalyst was filtered off on a cintered funnel and acetonitrile was removed in vacuo. The resulting residue was taken in EtOAc (25 mL) and washed successively with saturated solution of NaHCO_3 (2X10 mL), water (2X10 mL) and brine (1X10 mL). Separating the organic phase, drying (Na_2SO_4) and concentration in vacuo, yielded the corresponding oxirane containing peptide **10b** in high purity and yields (HPLC). This was further subjected to column chromatography (silica gel; EtOAc:Hexane - 2:3) for purification to get to the pure epoxide **10b** as a gum in excellent yields (84 %) $[\alpha]_D^{25} = -57^\circ$ ($c = 0.018$, CH_2Cl_2).

^1H NMR, 60 MHz, CDCl_3 , δ 7.50 - 7.36 (m, 5H), 7.25 (d, $J = 7$ Hz, 1H), 7.14 (s, 5H), 4.83 (dd, $J = 8.6$ Hz & 4 Hz, 1H), 4.70 (d, $J = 11$ Hz, 1H), 3.83 (d, $J = 5.1$ Hz, 1H), 3.67 (s, 3H), 3.62 (d, $J = 5.1$ Hz, 1H), 3.58 - 3.49 (m, 1H), 3.48 - 3.39 (m, 1H), 3.10 (dd, $J = 11$ Hz & 4.5 Hz, 2H), 2.55 - 2.36 (m, 1H), 2.05 (s, 1H), 2.00 - 1.74 (m, 2H)

Synthesis of methyl-N-(3-phenylglycidyl)-proline-isoleucinate (10c)

To a solution of the methyl-N-cinnamoyl-proline-isoleucinate (632 mg, 1.7 mmol) in CH_3CN (8 mL) was added 2-methylpropanal (245 mg, 3.4 mmol) and PASCOS catalyst (~5 mg) and the contents were stirred under oxygen atmosphere at RT for 12 h. After this time, a fresh lot of the catalyst and 2-methylpropanal (245 mg, 3.4 mmol) were added to the reaction mixture and allowed to stir, until complete conversion of the olefin (TLC - R_f = 0.45; EtOAc:Hexane - 1:1). The catalyst was filtered off on a cintered funnel and acetonitrile was removed in vacuo. The resulting residue was taken in EtOAc (25 mL)

and washed successively with saturated solution of NaHCO_3 (2X10 mL), water (2X10 mL) and brine (1X10 mL). Separating the organic phase, drying (Na_2SO_4) and concentration in vacuo, yielded the corresponding oxirane containing peptide **10c** in high purity and yields (HPLC). This was further subjected to column chromatography (silica gel; EtOAc:Hexane - 2:3) for purification to get to the pure epoxide **10c** as a gum in excellent yields (80 %) $[\alpha]_D^{25} = -15^\circ$ ($c = 0.02$, CH_2Cl_2).

^1H NMR, 60 MHz, CDCl_3 , δ 7.50 (d, $J = 7$ Hz, 1H), 7.22 (s, 5H), 4.55 (t, $J = 7.5$ Hz, 1H), 4.43 (dd, $J = 9.5$ Hz & 6 Hz, 1H), 3.96 (d, $J = 4.1$ Hz, 1H), 3.78 – 3.70 (m, 1H), 3.61 (s, 3H), 3.54 (d, $J = 6.8$ Hz, 1H), 3.50 – 3.44 (m, 1H), 2.21 (d, $J = 8$ Hz, 1H), 2.18 – 1.95 (m, 2H), 1.90 – 1.73 (m, 1H), 1.15 (q, $J = 6.5$ Hz, 2H), 1.17 – 1.15 (m, 1H), 0.82 (d, $J = 8.8$ Hz, 6H); MS m/z 388, 373, 357, 307, 269, 257, 244, 209, 181, 154, 136

Synthesis of dimethyl-N-(3-phenylglycidyl)-proline-aspartate (**10d**)

To a solution of the methyl-N-cinnamoyl-proline-aspartate (776 mg, 2 mmol) in CH_3CN (10 mL) was added 2-methylpropanal (288 mg, 4 mmol) and PASCOS catalyst (~5 mg) and the contents were stirred under oxygen atmosphere at RT for 12 h. After this time, a fresh lot of the catalyst and 2-methylpropanal (288 mg, 4 mmol) were added to the reaction mixture and allowed to stir, until complete conversion of the olefin (TLC - $R_f = 0.5$; EtOAc:Hexane - 1:1). The catalyst was filtered off on a cindered funnel and acetonitrile was removed in vacuo. The resulting residue was taken in EtOAc (25 mL) and washed successively with saturated solution of NaHCO_3 (2X10 mL), water (2X10 mL) and brine (1X10 mL). Separating the organic phase, drying (Na_2SO_4) and concentration in vacuo, yielded the corresponding oxirane containing peptide **10d** in high purity and yields (HPLC). This was further subjected to column chromatography (silica gel; EtOAc:Hexane - 2:3) for purification to get to the pure epoxide **10d** as a gum in excellent yields (81 %) $[\alpha]_D^{25} = -72^\circ$ ($c = 0.02$, CH_2Cl_2).

^1H NMR, 400 MHz, CDCl_3 , δ 7.52 (d, $J = 8.3$ Hz, 1H), 7.35 – 7.32 (m, 5H), 4.85 (dd, $J = 8$ Hz & 4.1 Hz, 1H), 4.62 (t, $J = 3.6$ Hz, 1H), 4.14 & 4.08 (d, $J = 5.7$ Hz, 0.5H each), 3.82 (dt, $J = 9.8$ Hz & 4.2 Hz, 1H), 3.74 (s, 3H), 3.69 (s, 3H), 3.65 – 3.61 (m, 1H), 3.58 (d, $J = 5.7$ Hz, 1H), 2.92 (dd, $J = 20$ Hz & 15.1 Hz, 2H), 2.36 – 2.33 (m, 1H), 2.27 – 2.19 (m, 1H), 2.15 – 2.10 (m, 1H), 1.90 (bs, 1H)

Synthesis of Methyl-N-(3-phenylglycidyl)-leucine-prolinate (**11a**)

To a solution of the methyl-N-cinnamoyl-leucine-prolinate (558 mg, 1.5 mmol) in CH_3CN (7.5 mL) was added 2-methylpropanal (216 mg, 3 mmol) and PASCOS catalyst (~5 mg) and the contents were stirred under oxygen atmosphere at RT for 12 h. After this time, a fresh lot of the catalyst and 2-methylpropanal (216 mg, 3 mmol) were added to the reaction mixture and allowed to stir, until complete conversion of the olefin (TLC - $R_f = 0.5$; EtOAc:Hexane - 2:3). The catalyst was filtered off on a cindered funnel and acetonitrile was removed in vacuo. The resulting residue was taken in EtOAc (25 mL) and washed successively with saturated solution of NaHCO_3 (2X10 mL), water (2X10 mL) and brine (1X10 mL). Separating the organic phase, drying (Na_2SO_4) and concentration in vacuo, yielded the corresponding oxirane containing peptide **11a** in high purity and yields (HPLC). This was further subjected to column chromatography (silica gel; EtOAc:Hexane - 2:3) for purification to get to the pure epoxide **11a** in good yields (72 %) $[\alpha]_D^{25} = -54^\circ$ ($c = 0.01$, CH_2Cl_2).

¹H NMR, 300 MHz, CDCl₃, δ 7.34 (s, 3H), 7.27 (s, 2H), 6.96-6.80 (dt, J = 14.4 Hz & J = 6.7 Hz, 1H), 4.52 (t, J = 3.9 Hz, 1H), 3.87 (bs, 1H), 3.73 (s, 3H), 3.65 (m, 1H), 3.50 (dt, J = 6.5 Hz & J = 3 Hz, 1H), 2.21-2.09 (m, 1H), 2.07-1.96 (m, 2H), 1.82-1.62 (m, 1H), 1.58 (t, J = 1.5 Hz, 1H), 1.52 (t, J = 6 Hz, 1H), 0.99 (d, J = 6 Hz, 3H), 0.95 (dd, J = 9 Hz & J = 3 Hz, 3H)

Synthesis of Methyl-N-(3-phenylglycidyl)-leucine-(4-*trans*-acetox)-prolinate (11b)

To a solution of the methyl-N-cinnamoyl-leucine-(4-*trans*-acetox)-prolinate (430 mg, 1 mmol) in CH₃CN (5 mL) was added 2-methylpropanal (144 mg, 2 mmol) and PASCOS catalyst (~5 mg) and the contents were stirred under oxygen atmosphere at RT for 12 h. After this time, a fresh lot of the catalyst and 2-methylpropanal (144 mg, 2 mmol) were added to the reaction mixture and allowed to stir, until complete conversion of the olefin (TLC - R_f = 0.45; EtOAc:Hexane - 3:2). The catalyst was filtered off on a cindered funnel and acetonitrile was removed in vacuo. The resulting residue was taken in EtOAc (25 mL) and washed successively with saturated solution of NaHCO₃ (2X10 mL), water (2X10 mL) and brine (1X10 mL). Separating the organic phase, drying (Na₂SO₄) and concentration in vacuo, yielded the corresponding oxirane containing peptide **11b** in high purity and yields (HPLC). This was further subjected to column chromatography (silica gel; EtOAc:Hexane - 2:3) for purification to get to the pure epoxide **11b** in good yields (78 %) [α]_D²⁵ = -124° (c = 0.015, CH₂Cl₂).

¹H NMR, 60 MHz, CDCl₃, δ 7.53 (d, J = 8 Hz, 1H), 7.32 (s, 5H), 5.25 (m, 1H), 4.75 (dd, J = 1.6 Hz & J = 5.2 Hz, 1H), 4.48 (t, J = 8 Hz, 1H), 4.50-4.37 (m, 1H), 4.16 (d, J = 6.5 Hz, 1H), 3.98 (d, J = 4.5 Hz, 1H), 3.80-3.68 (m, 1H), 3.60 (s, 3H), 3.46 (d, J = 4.45 Hz, 1H), 2.22 (dd, J = 7.6 Hz & J = 3.2 Hz, 2H), 2.00 (s, 3H), 1.66-1.40 (m, 3H), 0.92 (d, J = 6.6 Hz, 6H); MS m/z 447 (M⁺), 401, 327, 281, 267, 251, 221, 207, 188, 154, 136

Synthesis of Methyl-N-(3-phenylglycidyl)-valine-prolinate (11c)

To a solution of the methyl-N-cinnamoyl-proline-leucinate (413 mg, 1.2 mmol) in CH₃CN (7 mL) was added 2-methylpropanal (173 mg, 2.4 mmol) and PASCOS catalyst (~5 mg) and the contents were stirred under oxygen atmosphere at RT for 12 h. After this time, a fresh lot of the catalyst and 2-methylpropanal (173 mg, 2.4 mmol) were added to the reaction mixture and allowed to stir, until complete conversion of the olefin (TLC - R_f = 0.5; EtOAc:Hexane - 3:2). The catalyst was filtered off on a cindered funnel and acetonitrile was removed in vacuo. The resulting residue was taken in EtOAc (25 mL) and washed successively with saturated solution of NaHCO₃ (2X10 mL), water (2X10 mL) and brine (1X10 mL). Separating the organic phase, drying (Na₂SO₄) and concentration in vacuo, yielded the corresponding oxirane containing peptide **11c** in high purity and yields (HPLC). This was further subjected to column chromatography (silica gel; EtOAc:Hexane - 2:3) for purification to get to the pure epoxide **11c** as a gum in excellent yields (92 %) [α]_D²⁵ = -57° (c = 0.005, CH₂Cl₂).

¹H NMR, 80 MHz, CDCl₃, δ 7.49-7.13 (m, 5H), 6.19 (d, J = 8.8 Hz, 1H), 4.98 (dd, J = 2.8 Hz & J = 5.1 Hz, 1H), 4.45 (dd, J = 11.2 Hz & 4.8 Hz, 1H), 3.87 (d, J = 6.6 Hz, 1H), 3.65 (d, J = 4.9 Hz, 1H), 3.55 (s, 3H), 3.41 (dd, J = 8 Hz & 4.2 Hz, 1H), 3.34 (d, J = 6.6 Hz, 1H), 2.44-2.03 (m, 2H), 2.00-1.97 (m, 1H), 1.90-1.8 (m, 1H), 0.91 (d, J = 6.6 Hz, 6H)

Synthesis of Methyl-N-(3-phenylglycidyl)-valine-(4-*trans*-acetoxy)-prolinate (11d)

To a solution of the methyl-N-cinnamoyl-proline-leucinate (744 mg, 2 mmol) in CH_3CN (10 mL) was added 2-methylpropanal (288 mg, 4 mmol) and PASCOS catalyst (~5 mg) and the contents were stirred under oxygen atmosphere at RT for 12 h. After this time, a fresh lot of the catalyst and 2-methylpropanal (288 mg, 4 mmol) were added to the reaction mixture and allowed to stir, until complete conversion of the olefin (TLC - R_f = 0.5; EtOAc:Hexane - 1:1). The catalyst was filtered off on a cindered funnel and acetonitrile was removed in vacuo. The resulting residue was taken in EtOAc (25 mL) and washed successively with saturated solution of NaHCO_3 (2X10 mL), water (2X10 mL) and brine (1X10 mL). Separating the organic phase, drying (Na_2SO_4) and concentration in vacuo, yielded the corresponding oxirane containing peptide **11d** in high purity and yields (HPLC). This was further subjected to column chromatography (silica gel; EtOAc:Hexane - 2:3) for purification to get to the pure epoxide **11d** as an oil in good yields (56 %).

^1H NMR, 80 MHz, CDCl_3 , δ 7.6-7.22(s, 5H), 6.78(dd, J = 6.1Hz & 5.6Hz, 1H), 5.26-5.00(m, 1H), 4.44(dd, J = 11.2Hz & 2.4Hz, 1H), 4.31(dd, J = 10.4Hz & J = 6.6Hz, 1H), 3.73 (dd, J = 5.6Hz & 3.2Hz, 1H), 3.67(d, J = 2.6Hz, 1H), 3.53(s, 3H), 3.48(d, J = 4.8Hz, 1H), 3.31(d, J = 2.6Hz, 1H), 2.09(dt, J = 6.7Hz & 3.2Hz, 2H), 1.84(s, 3H), 1.77-1.64(m, 1H), 0.74(d, J = 8.8Hz, 6H)

Synthesis of Methyl-N-(3-phenylglycidyl)-valine-(4-*trans*-hydroxy)-prolinate (11e)

To a solution of the methyl-N-cinnamoyl-valine-(4-*trans*-hydroxy)prolinate (784 mg, 2 mmol) in CH_3CN (10 mL) was added 2-methylpropanal (288 mg, 4 mmol) and PASCOS catalyst (~5 mg) and the contents were stirred under oxygen atmosphere at RT for 12 h. After this time, a fresh lot of the catalyst and 2-methylpropanal (288 mg, 4 mmol) were added to the reaction mixture and allowed to stir, until complete conversion of the olefin (TLC - R_f = 0.5; EtOAc:Hexane - 1:1). The catalyst was filtered off on a cindered funnel and acetonitrile was removed in vacuo. The resulting residue was taken in EtOAc (25 mL) and washed successively with saturated solution of NaHCO_3 (2X10 mL), water (2X10 mL) and brine (1X10 mL). Separating the organic phase, drying (Na_2SO_4) and concentration in vacuo, yielded the corresponding oxirane containing peptide **11e**. This was further subjected to column chromatography (silica gel; EtOAc:Hexane - 2:3) for purification to get to the pure epoxide **11e** in moderate yields (48 %).

^1H NMR, 80 MHz, CDCl_3 , δ 7.26(d, J = 8Hz, 1H), 7.12(s, 5H), 4.45(t, J = 9.6Hz, 1H), 4.38(t, J = 6.5Hz, 1H), 4.24-4.16(m, 1H), 3.8(dd, J = 14.4Hz & 9.6Hz, 1H), 3.76(bs, 1H), 3.56(s, 3H), 3.39(bs, 1H), 2.09(dt, J = 17.6Hz & 8Hz, 2H), 1.98-1.88 (m, 1H), 0.78 (d, J = 8Hz, 6H)

Synthesis of Methyl-3-(*m*-chlorophenyl)glycidyl-phenylalanine-prolinate (11f)

To a solution of the Methyl-N-(3'-chlorocinnamoyl)-phenylalanine-prolinate (883 mg, 2 mmol) in CH_3CN (10 mL) was added 2-methylpropanal (288 mg, 4 mmol) and PASCOS catalyst (~5 mg) and the contents were stirred under oxygen atmosphere at RT for 12 h. After this time, a fresh lot of the catalyst and 2-methylpropanal (288 mg, 4 mmol) were added to the reaction mixture and allowed to stir, until complete conversion of the olefin

(TLC - R_f = 0.45; EtOAc:Hexane - 1:1). The catalyst was filtered off on a cindered funnel and acetonitrile was removed in vacuo. The resulting residue was taken in EtOAc (25 mL) and washed successively with saturated solution of NaHCO_3 (2X10 mL), water (2X10 mL) and brine (1X10 mL). Separating the organic phase, drying (Na_2SO_4) and concentration in vacuo, yielded the corresponding oxirane containing peptide **11f** in high purity and yields (HPLC). This was further subjected to column chromatography (silica gel; EtOAc:Hexane - 2:3) for purification to get to the pure epoxide **11f** as a gum in excellent yields (89 %).

^1H NMR, 300MHz, CDCl_3 , δ 7.36(d, J = 6 Hz, 1H), 7.28 – 7.00 (m, 9H), 5.02 (t, J = 9 Hz, 1H), 4.46 (dd, J = 15 Hz & 8.6 Hz, 1H), 4.06 (dd, J = 7.8 Hz & 4 Hz, 1H), 3.75(d, J = 2.4 Hz, 1H), 3.66 (s, 3H), 3.60 (d, J = 2.4 Hz, 1H), 3.45(dd, J = 6Hz & 1.5 Hz, 1H), 3.08 (dd, J = 6 Hz & 1.5 Hz, 1H), 2.16 (bs, 1H), 2.10 – 1.97 (m, 1H), 1.95 – 1.86 (m, 1H), 1.87 – 1.76 (m, 1H); MS m/z - 457(M^+), 305, 277

Synthesis of methyl-N-(3-phenylglycidyl)-(O-acetyl)-tyrosinate (16)

To a solution of the methyl-N-cinnamoyl-(O-acetyl)-tyrosinate (368 mg, 1 mmol) in CH_3CN (5 mL) was added 2-methylpropanal (144 mg, 2 mmol) and PASCOS catalyst (~5 mg) and the contents were stirred under oxygen atmosphere at RT for 12 h. After this time, a fresh lot of the catalyst and 2-methylpropanal (144 mg, 2 mmol) were added to the reaction mixture and allowed to stir, until complete conversion of the olefin (TLC - R_f = 0.5; EtOAc:Hexane - 2:3). The catalyst was filtered off on a cindered funnel and acetonitrile was removed in vacuo. The resulting residue was taken in EtOAc (25 mL) and washed successively with saturated solution of NaHCO_3 (2X10 mL), water (2X10 mL) and brine (1X10 mL). Separating the organic phase, drying (Na_2SO_4) and concentration in vacuo, yielded the corresponding oxirane containing peptide **16** in high purity and yields (HPLC). This was further subjected to column chromatography (silica gel; EtOAc:Hexane - 1:2) for purification to get to the pure epoxide **16** as a gum in excellent yields (78 %).

^1H NMR, 60 MHz, CDCl_3 , δ 7.84(d, J = 8.8Hz, 1H), 7.55-7.25(m, 5H), 7.03(d, J = 3.8Hz, 2H), 6.96(d, J = 3.8Hz, 2H), 4.83(dt, J = 19.2Hz & J = 8.8Hz, 1H), 3.84(d, J = 2.4Hz, 1H), 3.59(s, 3H), 3.44(d, J = 2.4Hz, 1H), 3.00(t, J = 7.2Hz, 2H), 2.16(s, 3H)

Synthesis of methyl-N-(3-phenylglycidyl)-(O-crotonoyl)-tyrosinate (17)

To a solution of the methyl-N-cinnamoyl-(O-crotonoyl)-tyrosinate (394 mg, 1 mmol) in CH_3CN (5 mL) was added 2-methylpropanal (144 mg, 2 mmol) and PASCOS catalyst (~5 mg) and the contents were stirred under oxygen atmosphere at RT for 12 h. After this time, a fresh lot of the catalyst and 2-methylpropanal (144 mg, 2 mmol) were added to the reaction mixture and allowed to stir, until complete conversion of the olefin (TLC - R_f = 0.5; EtOAc:Hexane - 1:1). The catalyst was filtered off on a cindered funnel and acetonitrile was removed in vacuo. The resulting residue was taken in EtOAc (25 mL) and washed successively with saturated solution of NaHCO_3 (2X10 mL), water (2X10 mL) and brine (1X10 mL). Separating the organic phase, drying (Na_2SO_4) and concentration in vacuo, yielded the corresponding oxirane containing peptide **17** in high purity and yields (HPLC). This was further subjected to column chromatography (silica gel; EtOAc:Hexane - 2:3) for purification to get to the pure epoxide **17** as a gum in excellent yields (86 %).

^1H NMR, CDCl_3 , 400 MHz, δ , 7.33 - 7.24 (m, 3H), 7.11 (t, $J = 9.2$ Hz, 4H), 7.00 (d, $J = 8.4$ Hz, 1H), 6.96 (d, $J = 8.4$ Hz, 1H), 5.9 (d, $J = 11$ Hz, 1H), 4.84 - 4.82 (m, 1H), 4.19 & 3.85 (bs, 1H), 3.64 (s, 3H), 3.47 & 3.41 (s, 1H), 3.05 - 3.00 (m, 2H), 1.86 (bs, 3H)

Synthesis of methyl-N-(3-phenylglycidyl)-leucinate (**21a**)

To a solution of the methyl-N-cinnamoyl-leucinate (550 mg, 2 mmol) in CH_3CN (10 mL) was added 2-methylpropanal (288 mg, 4 mmol) and PASCOS catalyst (~5 mg) and the contents were stirred under oxygen atmosphere at RT for 12 h. After this time, a fresh lot of the catalyst and 2-methylpropanal (288 mg, 4 mmol) were added to the reaction mixture and allowed to stir, until complete conversion of the olefin (TLC - $R_f = 0.5$; EtOAc:Hexane - 1:1). The catalyst was filtered off on a cintered funnel and acetonitrile was removed in vacuo. The resulting residue was taken in EtOAc (25 mL) and washed successively with saturated solution of NaHCO_3 (2X10 mL), water (2X10 mL) and brine (1X10 mL). Separating the organic phase, drying (Na_2SO_4) and concentration in vacuo, yielded the corresponding oxirane containing peptide **21a** in high purity and yields (HPLC). This was further subjected to column chromatography (silica gel; EtOAc:Hexane - 2:3) for purification to get to the pure epoxide **21a** as a gum in excellent yields (88 %) $[\alpha]_D^{25} = +8^\circ$ ($c = 0.001$, CH_2Cl_2).

^1H NMR 60 MHz, CCl_4 , δ 7.69 - 7.28 (m, 5H), 4.92 - 4.76 (m, 1H), 3.94 (d, $J = 2.3$ Hz, 1H), 3.8 (s, 3H), 3.32 (d, $J = 2.3$ Hz, 1H), 1.65 - 1.45 (m, 2H), 1.35 - 1.30 (m, 1H), 1.10 (d, $J = 6$ Hz, 6H); IR (Neat): ν_{max} 3430 - 3290 (br), 1730, 1640, 1520 cm^{-1} .

Synthesis of Methyl-N-(3-phenylglycidyl)-valinate (**21b**)

To a solution of the methyl-N-cinnamoyl-valinate (522 mg, 2 mmol) in CH_3CN (10 mL) was added 2-methylpropanal (288 mg, 4 mmol) and PASCOS catalyst (~5 mg) and the contents were stirred under oxygen atmosphere at RT for 12 h. After this time, a fresh lot of the catalyst and 2-methylpropanal (288 mg, 4 mmol) were added to the reaction mixture and allowed to stir, until complete conversion of the olefin (TLC - $R_f = 0.5$; EtOAc:Hexane - 1:3.3). The catalyst was filtered off on a cintered funnel and acetonitrile was removed in vacuo. The resulting residue was taken in EtOAc (25 mL) and washed successively with saturated solution of NaHCO_3 (2X10 mL), water (2X10 mL) and brine (1X10 mL). Separating the organic phase, drying (Na_2SO_4) and concentration in vacuo, yielded the corresponding oxirane containing peptide **21b** in high purity and yields (HPLC). This was further subjected to column chromatography (silica gel; EtOAc:Hexane - 1:4) for purification to get to the pure epoxide **21b** as a gum in excellent yields (87).

^1H NMR 60 MHz, CDCl_3 , δ 7.7 - 7.20 (m, 5H), 4.72 - 4.43 (m, 1H), 3.93 (d, $J = 4$ Hz, 1H), 3.75 (s, 3H), 3.5 (d, $J = 4$ Hz, 1H), 2.3 - 2.25 (m, 1H), 0.98 (d, $J = 6$ Hz, 6H); IR : ν_{max} 3430 - 3280 (br), 1735, 1660, 1520 cm^{-1}

Synthesis of methyl-N-(3-phenylglycidyl)-phenylalaninate (**21c**)

To a solution of methyl-N-cinnamoyl-phenylalaninate (620 mg, 2 mmol) in CH_3CN (10 mL) was added 2-methylpropanal (288 mg, 4 mmol) and PASCOS catalyst (~5 mg) and the contents were stirred under oxygen atmosphere at RT for 12 h. After this time, a fresh lot of the catalyst and 2-methylpropanal (288 mg, 4 mmol) were added to the reaction mixture and allowed to stir, until complete conversion of the olefin (TLC - $R_f = 0.4$; EtOAc:Hexane - 1:3). The catalyst was filtered off on a cintered funnel and acetonitrile was removed in vacuo. The resulting residue was taken in EtOAc (25 mL) and washed

successively with saturated solution of NaHCO_3 (2X10 mL), water (2X10 mL) and brine (1X10 mL). Separating the organic phase, drying (Na_2SO_4) and concentration in vacuo, yielded the corresponding oxirane containing peptide **21c** in high purity and yields (HPLC). This was further subjected to column chromatography (silica gel; EtOAc:Hexane - 1:3) for purification to get to the pure epoxide **21c** as solid in excellent yields (82 %).

^1H NMR 60 MHz, CDCl_3 , δ 7.52 - 6.78 (m, 10H), 6.72 (bs, 1H), 4.82 - 4.69 (m, 1H), 3.8 (d, $J = 2.5$ Hz, 1H), 3.7 (s, 3H), 3.15 (d, $J = 2.5$ Hz, 1H), 3.0 (d, $J = 6$ Hz, 2H); IR (Neat): ν_{max} 3330 - 3280 (br), 1735, 1640, 1520 cm^{-1}

Synthesis of methyl-N-(3-phenylglycidyl)-prolinate (**23**)

To a solution of methyl-N-cinnamoyl-prolinate (518 mg, 2 mmol) in CH_3CN (10 mL) was added 2-methylpropanal (288 mg, 4 mmol) and PASCOS catalyst (~5 mg) and the contents were stirred under oxygen atmosphere at RT for 12 h. After this time, a fresh lot of the catalyst and 2-methylpropanal (288 mg, 4 mmol) were added to the reaction mixture and allowed to stir, until complete conversion of the olefin (TLC - $R_f = 0.5$; EtOAc:Hexane - 1:2). The catalyst was filtered off on a cindered funnel and acetonitrile was removed in vacuo. The resulting residue was taken in EtOAc (25 mL) and washed successively with saturated solution of NaHCO_3 (2X10 mL), water (2X10 mL) and brine (1X10 mL). Separating the organic phase, drying (Na_2SO_4) and concentration in vacuo, yielded the corresponding oxirane containing peptide **23** in high purity and yields (HPLC). This was further subjected to column chromatography (silica gel; EtOAc:Hexane - 2:3) for purification to get to the pure epoxide **23** as a gum in excellent yields (90 %) [α] $_{\text{D}}^{25} = -58^\circ$ ($c = 0.0018$, CH_2Cl_2).

^1H NMR 400 MHz, CDCl_3 , δ 7.36 - 7.27 (m, 5H), 4.63 (dt, $J = 8$ Hz & 3.6 Hz, 1H), 4.14 (s, 0.5H), 4.08 (s, 0.5H), 3.84 (dd, $J = 10.4$ Hz & 8 Hz, 1H), 3.74 (s, 3H), 3.61 (dd, $J = 3.6$ Hz & 0.8 Hz, 1H), 3.42 (s, 1H), 2.33 - 1.90 (m, 4H); IR (Neat) ν_{max} 3440 - 3280 (br), 1790, 1725, 1660, 1520 cm^{-1}

3-phenyl-(2S,3S)-oxy propanol (**24**)

Compound **24** was synthesised according to the Sharpless epoxidation procedure.³⁸⁹

^1H NMR, CDCl_3 , 400 MHz, δ 7.37 - 7.24 (m, 5H), 4.01 (dd, $J = 12.7$ Hz & 2.9 Hz, 1H), 3.90 (d, $J = 2.2$ Hz, 1H), 3.74 (dd, $J = 12$ Hz & 6.8 Hz, 1H), 3.21 (td, $J = 4.1$ Hz & 2.2 Hz, 1H), 2.71 (bs, 1H) [α] $_{\text{D}}^{25} = -49^\circ$ ($c = 0.01$, CH_2Cl_2)

Synthesis of methyl-3-phenyl-(2R,3S)-glycyl-prolinate (**26**)

$\text{Ru(III)Cl}_2 \cdot \text{H}_2\text{O}$ (7.5 mg, 33 μM) was added to a stirring biphasic mixture of the corresponding epoxy alcohol (150 mg, 1 mmol), sodium periodate (643 mg, 3 mmol) and sodium bicarbonate (420 mg, 5 mmol) in CCl_4 (2 mL), acetonitrile (2 mL) and water (3 mL). After 42 h of stirring additional amounts of RuCl_3 (7.6 mg, 34 μM) and sodiumperiodate (157 mg) were added and stirring continued for 1h to complete the reaction. Then dichloromethane (8 mL) was added and the small amount of water (until phase separation occurred). The pH of the water layer was adjusted to 4 and the aqueous layer was extracted with dichloromethane. Acidification and extraction were repeated until the pH remained constant. The combined layers were dried (Na_2SO_4) and taken in a clean dry flask. Triethylamine (0.2 mL, 1.5 mmol) was added to it and the reaction vessel was cooled to -5°C in a ice-salt bath. Isobutylchloroformate (0.13 mL, 1 mmol) was

added to it and stirred for 0.5 min. A solution of methyl-L-proline hydrochloride (248 mg, 1.5 mmol) in DMSO (0.5 mL) was added to it and stirred vigorously for 3-4 h. Removal of solvent under vacuum yielded a residue, which was taken in EtOAc and washed with saturated aqueous solution of NaHCO_3 , water and brine. The resulting organic layer was dried and concentrated to give a residue which was subjected to column chromatography to yield the required product as a gum in moderate yields (36 %) $[\alpha]_D^{25} = -203^\circ$ ($c = 0.001$, CH_2Cl_2).

^1H NMR, 400 MHz, CDCl_3 δ 7.40 - 7.18 (m, 5H), 4.55 (dd, $J = 8.3$ Hz & 4.4 Hz, 1H), 4.07 & 3.99 (d, $J = 2$ Hz, 1H), 3.86 - 3.80 (m, 1H), 3.73 (s, 3H), 3.65 - 3.61 (m, 1H), 3.59 (d, $J = 2$ Hz, 1H), 2.29 - 2.18 (m, 1H), 2.17 - 2.09 (m, 1H), 2.07 - 2.02 (m, 1H), 2.01 - 1.90 (m, 1H); IR (Neat) ν_{max} 3440 - 3280 (br), 1790, 1725, 1660, 1520 cm^{-1}

Synthesis of methyl-3-phenyl-(2R,3S)-glycyl-proline-leucinate (27)

$\text{Ru(III)Cl}_3 \cdot \text{H}_2\text{O}$ (7.5 mg, 33 μM) was added to a stirring biphasic mixture of the corresponding epoxy alcohol (150 mg, 1 mmol), sodium periodate (643 mg, 3 mmol) and sodium bicarbonate (420 mg, 5 mmol) in CCl_4 (2 mL), acetonitrile (2 mL) and water (3 mL). After 42 h of stirring additional amounts of RuCl_3 (7.6 mg, 34 μM) and sodiumperiodate (157 mg) were added and stirring continued for 1h to complete the reaction. Then dichloromethane (8 mL) was added and the small amount of water (until phase separation occurred). The pH of the water layer was adjusted to 4 and the aqueous layer was extracted with dichloromethane. Acidification and extraction were repeated until the pH remained constant. The combined layers were dried (Na_2SO_4) and taken in a clean dry flask. Triethylamine (0.2 mL, 1.5 mmol) was added to it and the reaction vessel was cooled to -5°C in a ice-salt bath. Isobutylchloroformate (0.13 mL, 1 mmol) was added to it and stirred for 0.5 min. A solution of methyl-L-proline-L-leucinate hydrochloride (418 mg, 1.5 mmol) in DMSO (0.5 mL) was added to it and stirred vigorously for 3-4 h. Removal of solvent under vacuum yielded a residue, which was taken in EtOAc and washed with saturated aqueous solution of NaHCO_3 , water and brine. The resulting organic layer was dried and concentrated to give a residue which was subjected to column chromatography to yield the required product as a solid in moderate yields (31 %, M.P. = $105-107^\circ\text{C}$) $[\alpha]_D^{25} = -191^\circ$ ($c = 0.0018$, CH_2Cl_2).

^1H NMR, CDCl_3 , 400 MHz, δ 7.30 - 7.26 (m, 3H), 7.25 - 7.20 (m, 2H), 7.11 (d, $J = 7.3$ Hz, 1H), 4.58 (dd, $J = 8.1$ Hz & 2 Hz, 1H), 4.41 (dd, $J = 13.2$ Hz & 7.8 Hz, 1H), 4.01 (d, $J = 1.44$ Hz, 1H), 3.70 (dd, $J = 8.5$ Hz & 3.6 Hz, 1H), 3.64 (s, 3H), 3.52 (t, $J = 1.1$ Hz, 1H), 3.48 (d, $J = 1.44$ Hz, 1H), 2.32 (tt, $J = 6.1$ Hz & 2.4 Hz, 1H), 2.12 - 2.05 (m, 1H), 1.94 - 1.75 (m, 2H), 1.69 - 1.45 (m, 2H), 1.16 (bs, 1H), 0.86 (d, $J = 5.6$ Hz, 3H), 0.84 (d, $J = 5.6$ Hz, 3H); MS m/z 388(M^+), 281, 269, 244, 209, 181, 154

Synthesis of methyl-N-(methyloxycarbonyl)-proline (28)

A stirring solution of methyl-L-proline hydrochloride (0.83 gm, 5 mmol) in THF (10 mL) and DMSO (2 mL) was cooled to -5°C and to it was added methyl chloroformate (0.39 mL) followed by drop wise addition of a solution of triethylamine (1.53 mL, 11 mmol) in THF (15 mL) at that temperature. The reaction mixture was warmed to room temperature after complete addition of the amine and further stirred for 2 h. Removal of solvent gave a residue which was taken in EtOAc (25 mL) and washed with a saturated aqueous solution of NaHCO_3 , water and brine. The resulting organic layer was dried and

concentrated in vacuo to give a gummy residue (0.719 gm), which was taken for hydrolysis.

Synthesis of methyl-N-(methyloxycarbonyl)-proline (29)

To a solution of methyl-(N-methyloxycarbonyl)-L-prolinate (0.719 gm, 4.1 mmol) in MeOH (8 mL) was added a solution of LiOH.H₂O (0.17 gm, 4.1 mmol) in water (2 mL) and the reaction mixture was stirred for 3 h. Removal of solvent yielded a residue to which was added CH₂Cl₂ (20 mL) followed by drop wise addition of 1N HCl while stirring, until complete acidification of the lithium salt. The organic layer was separated and washed with saturated aqueous solution of NaHCO₃, water and brine. The resulting organic layer was dried and concentrated to give the crude acid in good yields (583mg, 88%) as a gum.

Synthesis of methyl-N-(methyloxycarbonyl)-proline leucinate (30)

To a solution of N-methyloxycarbonyl-L-proline (0.583 mg, 3.6 mmol) in THF (7.5 mL) was added triethylamine (0.5 mL, 3.6 mmol) was added to it and the reaction vessel was cooled to -5 °C in a ice-salt bath. methylchloroformate (0.40 mL, 3.6 mmol) was added to it and stirred for 50-55 sec. A solution of methyl-L-leucinate hydrochloride (0.653 mg, 3.6 mmol) in DMSO (1.5 mL) was added to it and stirred vigorously for 3-4 h. Removal of solvent under vacuum yielded a residue, which was taken in EtOAc and washed with saturated aqueous solution of NaHCO₃, water and brine. The resulting organic layer was dried and concentrated to give a residue, which was subjected to column chromatography to yield the required product as a gum in good yields (0.691 gm, 2.4 mmol, 65 %).

Synthesis of methyl-proline-leucinate.hydrochloride (31)

Methyl-(N-methyloxycarbonyl)-L-proline-L-leucinate (0.69 gm, 2.4 mmol) was taken in a clean dry flask and to it was added a 4N HCl solution in MeOH (5 mL). The reaction mixture was stirred for 0.5 h. Removal of solvent yielded a residue, which was washed with ether (2 X 10 mL) and dried under vacuo for 3-4 h to yield the hydrochloride salt as a solid (M.P. 70-72 °C) in good yield (557 mg, 2 mmol).

β -Phenylisoserine Derived Peptides

General Procedure for the Hydroxyamination of N-cinnamoylpeptides

To the methyl-N-cinnamoyl-dipeptide (1 equivalent) in acetonitrile (5 mL/mmol), 2-methylpropanal (2 equivalents) and catalytic amounts of the polyaniline supported cobalt(II)salen (PASCOS) (~5mg) catalyst were added and the resulting solution was stirred under oxygen atmosphere for 12 h, after which a fresh lot of the catalyst and 2-methylpropanal (2 euivalents) were added and the reaction was stirred to completion (TLC - R_f = 0.5 - EtOAc:Hexane - 1.1). After complete conversion of the olefin to the glycidic amide, the oxygen balloon was removed and a fresh lot of catalyst was added to the reaction mixture followed by the aromatic amine (1 equivalent) of choice. After stirring until complete consumption of the epoxide and formation of the product (TLC - R_f = 0.5 - EtOAc:Hexane - 1.1), the catalyst was filtered under suction through a sintered funnel and washed with acetonitrile. The combined filtrate was concentrated in vacuum to yield a residue which was taken in minimum volume of CCl₄ and hexane was added to it drop wise. A dark brown residue precipitated. The mother liquor was decanted and the residue was washed repeatedly with CCl₄:hexane solvent mixture, until complete removal

of the unreacted aromatic amine, to get to the β -phenylisoserine derived peptide in high purity (HPLC) and good yields, mostly as good solids.

Synthesis of methyl-N-(*p*-methoxyphenyl)- β -phenylisoserine-proline-leucinate (3)

To a solution of methyl-N-cinnamoyl-proline-leucinate (744 mg, 2 mmol) in acetonitrile (10 mL), 2-methylpropanal (288 mg, 4 mmol) and PASCOS (~5mg) catalyst were added and the resulting solution was stirred under oxygen atmosphere for 12 h, after which a fresh lot of the catalyst and 2-methylpropanal (288 mg, 4 mmol) were added and the reaction was stirred to completion ((TLC - R_f = 0.5 - EtOAc:Hexane - 1:1)). After complete conversion of the olefin to the glycidic amide, the oxygen balloon was removed and a fresh lot of catalyst was added to the reaction mixture followed by *p*-anisidine (246 mg, 2 mmol). After stirring until complete consumption of the epoxide and formation of the product (TLC - R_f = 0.5 EtOAc:Hexane - 3:2), washing of the unreacted material as described in the general procedure, with CCl_4 :hexane gave the β -phenylisoserine derived peptide 3 in high purity (HPLC) and good yields (62 %), predominantly as the anti diastereomer.

^1H NMR 400 MHz, CDCl_3 , δ 7.39 - 7.15 (m, 5H), 6.84 (d, J = 7.6 Hz, 1H), 6.66 (d, J = 9 Hz, 2H), 6.51 (d, J = 9 Hz, 2H), 4.73 (d, J = 2.9 Hz, 1H), 4.64 (d, J = 3.2 Hz, 1H), 4.52 (d, J = 7.6 Hz, 1H), 4.48 (dd, J = 8 Hz & 4.1 Hz, 1H), 3.75 (s, 3H), 3.74 - 3.69 (m, 2H), 3.67 (s, 3H), 2.17 - 2.15 (m, 1H), 1.92 - 1.85 (m, 2H), 1.77 - 1.47 (m, 4H), 0.92 (d, J = 6.6 Hz, 3H), 0.89 (d, J = 6.5 Hz, 3H); FTIR (CH_2Cl_2): 3286.7, 2957.3, 2870.6, 1744.3, 1649.0, 1510.2, 1438, 1367.5, 1242

Synthesis of methyl-N-(*p*-methylphenyl)- β -phenylisoserine-leucine-(4-*trans*-acetoxy)prolinate (9b₁)

To a solution of methyl-N-cinnamoyl-leucine-(4-*trans*-acetoxy)prolinate (645 mg, 1.5 mmol) in acetonitrile (7.5 mL), 2-methylpropanal (216 mg, 3 mmol) and PASCOS (~5mg) catalyst were added and the resulting solution was stirred under oxygen atmosphere for 12 h, after which a fresh lot of the catalyst and 2-methylpropanal (216 mg, 3 mmol) were added and the reaction was stirred to completion (TLC - R_f = 0.5 - EtOAc:Hexane - 1:1). After complete conversion of the olefin to the glycidic amide, the oxygen balloon was removed and a fresh lot of catalyst was added to the reaction mixture followed by *p*-toluedine (160 mg, 1.5 mmol). After stirring until complete consumption of the epoxide and formation of the product (TLC - R_f = 0.6 - EtOAc:Hexane - 3:2), washing of the unreacted material as described in the general procedure, with CCl_4 :hexane gave the β -phenylisoserine derived peptide 9b₁ in high purity (HPLC) and good yields (59 %), predominantly as the anti diastereomer.

(anti-diastereomer) ^1H NMR, 300 MHz, CDCl_3 , δ 7.40 (d, J = 7.2 Hz, 1H), 7.35 - 7.14 (m, 5H), 6.91 (d, J = 8.1 Hz, 2H), 6.54 (d, J = 8.4 Hz, 2H), 5.39 - 5.38 (m, 1H), 4.77 (d, J = 4.5 Hz, 1H), 4.81 (d, J = 4.5 Hz, 1H), 4.70 - 4.62 (m, 1H), 4.54 (t, J = 8.4 Hz, 1H), 4.49 - 4.43 (m, 1H), 3.86 - 3.83 (m, 1H), 3.71 (s, 3H), 3.58 - 3.44 (m, 1H), 2.5 - 2.34 (m, 2H), 2.18 (s, 3H), 2.08 (s, 3H), 1.56 - 1.50 (m, 1H), 1.47 - 1.41 (m, 2H), 0.94 (d, J = 6Hz, 3H), 0.88 (dd, J = 6.3 Hz & 2.7 Hz, 3H); MS m/z 553(M^+), 357, 327, 268, 196, 143, 91; IR: ν_{max} : 3380(s), 3300, 3220, 2930, 1730, 1660, 1600 cm^{-1}

(syn-diastereomer) ^1H NMR, 300 MHz, CDCl_3 , δ 7.40 - 7.35 (m, 3H), 7.20 - 7.21 (m, 3H), 6.90 (d, J = 9 Hz, 2H), 6.56 (d, J = 6Hz, 2H), 4.89 - 4.86 (m, 1H), 4.87 (dd, J = 3 Hz

& 6 Hz, 2H), 4.59 - 4.50 (m, 3H), 3.99 - 3.96 (m, 1H), 3.85 - 3.81 (m, 1H), 3.73 (s, 3H), 2.37 - 2.48 (m, 2H), 2.18 (s, 3H), 2.05 (s, 3H), 1.32 - 1.26 (m, 1H), 1.24 - 1.14 (m, 1H), 1.00 - 0.89 (m, 1H), 0.78 (dd, $J = 9$ Hz & 6 Hz, 3H), 0.72 (t, $J = 6$ Hz, 3H); MS m/z : 553, 357, 327, 268, 196, 143, 91

Synthesis of methyl-N-(*p*-methoxyphenyl)- β -phenylisoserine-leucine-(4-*trans*-acetoxy)prolinate (**9b₂**)

To a solution of methyl-N-cinnamoyl-leucine-(4-*trans*-acetoxy)prolinate (642 mg, 1.5 mmol) in acetonitrile (7.5 mL), 2-methylpropanal (216 mg, 3 mmol) and PASCOS (~5mg) catalyst were added and the resulting solution was stirred under oxygen atmosphere for 12 h, after which a fresh lot of the catalyst and 2-methylpropanal (216 mg, 3 mmol) were added and the reaction was stirred to completion (TLC - $R_f = 0.5$ - EtOAc:Hexane - 1:1). After complete conversion of the olefin to the glycidic amide, the oxygen balloon was removed and a fresh lot of catalyst was added to the reaction mixture followed by *p*-anisidine (185 mg, 1.5 mmol). After stirring until complete consumption of the epoxide and formation of the product (TLC - $R_f = 0.5$ - EtOAc:Hexane - 3:2), washing of the unreacted material as described in the general procedure, with CCl₄:hexane gave the β -phenylisoserine derived peptide **9b₂** in high purity (HPLC) and good yields (62 %), predominantly as the anti diastereomer.

(anti-diastereomer) ¹H NMR, 300 MHz, CDCl₃, δ 7.36 - 7.34 (m, 2H), 7.33 - 7.24 (m, 3H), 6.94 (d, $J = 9.3$ Hz, 1H), 6.70 (d, $J = 12$ Hz, 2H), 6.59 (d, $J = 12$ Hz, 2H), 5.36 (m, 1H), 4.71 (dd, $J = 10$ Hz & 5.1 Hz, 1H), 4.44 (t, $J = 2.4$ Hz, 1H), 4.39 (dd, $J = 8.1$ Hz & 3 Hz, 1H), 3.70 (s, 3H), 3.69 (s, 3H), 3.62 - 3.44 (m, 2H), 2.02 - 1.94 (s, 3H), 1.57 - 1.50 (m, 3H), 1.47 (d, $J = 4.5$ Hz, 1H), 1.17 (d, $J = 6$ Hz, 1H), 0.95 (d, $J = 6$ Hz, 3H), 0.89 (dd, $J = 6.3$ Hz & 4.5 Hz, 3H); MS m/z : 569(M⁺), 357, 327, 212, 187, 107; IR: ν_{\max} 3400, 3355, 3060, 2850, 1735, 1600 cm⁻¹

(syn-diastereomer) ¹H NMR, 300 MHz, CDCl₃, δ 7.36 - 7.33 (m, 2H), 7.27 - 7.17 (m, 3H), 6.98 (d, $J = 8.6$ Hz, 1H), 6.67 (d, $J = 8.8$ Hz, 2H), 6.59 (d, $J = 8.8$ Hz, 2H), 5.36 - 5.33 (m, 1H), 4.82 (dd, $J = 6.3$ Hz & 3.7 Hz, 1H), 4.60 - 4.55 (m, 2H), 4.48 (t, $J = 8.1$ Hz, 1H), 3.98 (dd, $J = 4.9$ Hz & 12 Hz, 1H), 3.82 (dd, $J = 11.3$ Hz & 4.9 Hz, 1H), 3.70 (s, 3H), 3.67 (s, 3H), 2.39 - 2.37 (m, 1H), 0.96 - 0.88 (m, 2H), 0.76 (t, $J = 6.1$ Hz, 3H), 0.702 (dd, $J = 9$ Hz, 6.6 Hz, 3H); MS m/z : 569(M⁺), 357, 327, 212, 187, 107;

Synthesis of methyl-N-(*p*-bromophenyl)- β -phenylisoserine-leucine-(4-*trans*-acetoxy)prolinate (**9b₃**)

To a solution of methyl-N-cinnamoyl-leucine-(4-*trans*-acetoxy)prolinate (645 gm, 1.5 mmol) in acetonitrile (7.5 mL), 2-methylpropanal (216 mg, 3 mmol) and PASCOS (~5mg) catalyst were added and the resulting solution was stirred under oxygen atmosphere for 12 h, after which a fresh lot of the catalyst and 2-methylpropanal (216 mg, 3 mmol) were added and the reaction was stirred to completion (TLC - $R_f = 0.5$ - EtOAc:Hexane - 1:1). After complete conversion of the olefin to the glycidic amide, the oxygen balloon was removed and a fresh lot of catalyst was added to the reaction mixture followed by *p*-bromoaniline (259 mg, 1.5 mmol). After stirring until complete consumption of the epoxide and formation of the product (TLC - $R_f = 0.4$ - EtOAc:Hexane - 1:1), washing of the unreacted material as described in the general

^1H NMR, CDCl_3 , 300 MHz, δ 7.83 - 7.71 (m, 1H), 7.56 (d, J = 4.2 Hz, 1H), 7.40 - 7.36 (m, 2H), 7.26 - 7.16 (m, 7H), 7.12 (d, J = 6.3 Hz, 2H), 6.90 (d, J = 8.4 Hz, 2H), 6.49 (d, J = 8.4 Hz, 2H), 4.85 - 4.73 (m, 3H), 4.39 (d, J = 7.5 Hz, 1H), 3.73 (s, 3H), 3.56 - 3.51 (m, 2H), 3.32 - 3.12 (m, 2H), 2.99 - 2.88 (m, 2H), 2.44 - 2.42 (m, 1H), 2.17 (s, 3H), 1.97 - 1.94 (m, 1H); MS m/z : 529 (M^+), 333, 196 IR: ν_{max} 3300, 3030, 2900, 1715, 1675 cm^{-1}

Synthesis of methyl - N - (*p*-methoxyphenyl) - β - phenylisoserine- proline - phenylalaninate (9c₂**)**

To a solution of methyl-N-cinnamoyl-proline- phenylalaninate (610 mg, 1.5 mmol) in acetonitrile (7.5 mL), 2-methylpropanal (216 mg, 3 mmol) and PASCOS (~5mg) catalyst were added and the resulting solution was stirred under oxygen atmosphere for 12 h, after which a fresh lot of the catalyst and 2-methylpropanal (216 mg, 3 mmol) were added and the reaction was stirred to completion (TLC - R_f = 0.45 - EtOAc:Hexane - 1:1). After complete conversion of the olefin to the glycidic amide, the oxygen balloon was removed and a fresh lot of catalyst was added to the reaction mixture followed by *p*-anisidine (184 mg, 1.5 mmol). After stirring until complete consumption of the epoxide and formation of the product (TLC - R_f = 0.5 - EtOAc:Hexane - 3:2), washing of the unreacted material as described in the general procedure, with CCl_4 :hexane gave the β -phenylisoserine derived peptide **9c₂** in high purity (HPLC) and good yields (56 %), predominantly as the anti diastereomer.

(anti-diastereomer) ^1H NMR, CDCl_3 , 300 MHz, δ 7.53 (dd, J = 5.4 Hz & 3 Hz, 1H), 7.42 (dd, J = 5.4 Hz & 2.4 Hz, 2H), 7.38 - 7.24 (m, 6H), 7.11 (d, J = 6.6 Hz, 1H), 6.84 (d, J = 9.9 Hz, 1H), 6.71 (d, J = 5.1 Hz, 2H), 6.55 (d, J = 6.3 Hz, 2H), 4.80 - 4.76 (m, 1H), 4.70 (dd, J = 13.8 Hz & 4.2 Hz, 1H), 4.55 (dd, J = 10.2 Hz & 4.5 Hz, 1H), 4.37 (d, J = 7.2 Hz, 1H), 4.08 (d, J = 6.6 Hz, 1H), 3.83 - 3.78 (m, 1H), 3.76 (s, 3H), 3.68 (s, 3H), 3.25 - 3.50 (m, 1H), 3.13 (dd, J = 14.1 Hz & 8.4 Hz, 1H), 2.83 - 2.92 (m, 1H), 2.43 - 2.39 (m, 2H), 2.20 - 2.14 (m, 2H); MS m/z : (M^+ = 545 absent), 212, 149; IR: ν_{max} : 3390(br), 3300, 3080, 2880, 1660, 1600 cm^{-1}

(sys diastereomer) ^1H NMR, CDCl_3 , 300 MHz, δ 7.40 (d, J = 6.9 Hz, 2H), 7.25 - 7.14 (m, 8H), 7.10 (d, J = 6.6 Hz, 2H), 6.98 (6s, 1H), 6.67 (d, J = 9.6 Hz, 2H), 4.78 (dd, J = 10.2 Hz & 4.2 Hz, 1H), 4.41 (t, J = 6 Hz, 1H), 4.32 - 4.18 (m, 1H), 4.08 (d, J = 6.6 Hz, 1H), 3.80 - 3.70 (m, 1H), 3.68 (s, 3H), 3.61 (s, 3H), 3.49 - 3.46 (m, 1H), 3.23 - 3.20 (m, 1H), 3.08 - 3.02 (m, 2H), 2.28 (d, J = 9.6 Hz, 1H), 2.18 (d, J = 9.3 Hz, 1H), 2.08 - 2.01 (m, 2H); MS m/z : 545(M^+), 438, 212, 149

Synthesis of methyl - N - (*p*-bromophenyl)- β - phenylisoserine - proline - phenylalaninate (9c₃**)**

To a solution of methyl-N-cinnamoyl-proline- phenylalaninate (610 mg, 1.5 mmol) in acetonitrile (7.5 mL), 2-methylpropanal (216 mg, 3 mmol) and PASCOS (~5mg) catalyst were added and the resulting solution was stirred under oxygen atmosphere for 12 h, after which a fresh lot of the catalyst and 2-methylpropanal (216 mg, 3 mmol) were added and the reaction was stirred to completion (TLC - R_f = 0.45 - EtOAc:Hexane - 1:1). After complete conversion of the olefin to the glycidic amide, the oxygen balloon was removed and a fresh lot of catalyst was added to the reaction mixture followed by *p*-bromoaniline (260 mg, 1.5 mmol). After stirring until complete consumption of the epoxide and formation of the product (TLC - R_f = 0.5 - EtOAc:Hexane - 1:1.22), washing of the unreacted material as described in the general procedure, with CCl_4 :hexane gave the β -

phenylisoserine derived peptide **9c₃** in high purity (HPLC) and good yields (50 %), predominantly as the anti diastereomer.

^1H NMR, CDCl_3 , 300 MHz, δ 7.37 (d, J = 8.4 Hz, 2H), 7.31 - 7.13 (m, 6H), 7.06 (d, J = 7.5 Hz, 2H), 6.99 (d, J = 8.4 Hz, 2H), 6.68 (d, J = 7.5 Hz, 1H), 6.50 (d, J = 8.4 Hz, 2H), 4.86 - 4.69 (m, 3H), 4.35 (dd, J = 10.2 Hz & 3 Hz, 1H), 3.77 (m, 1H), 3.69 (s, 3H), 3.44 - 4.42 (m, 1H), 3.06 - 3.05 (m, 1H), 3.00 (dd, J = 13.6 Hz & 6.0 Hz, 2H), 2.94 (m, 1H), 2.17 (s, 3H), 1.90 (sextet, J = 7.2 Hz, 2H); IR: ν_{max} 3340, 3230, 2900, 1710, 1655, 1625 cm^{-1} .

Synthesis of methyl - N - (*p*-methylphenyl) - β - phenylisoserine - leucine - proline (9d₁)

To a solution of methyl-N-cinnamoyl-leucine-proline (558 mg, 1.5 mmol) in acetonitrile (7.5 mL), 2-methylpropanal (216 mg, 3 mmol) and PASCOS (~5mg) catalyst were added and the resulting solution was stirred under oxygen atmosphere for 12 h, after which a fresh lot of the catalyst and 2-methylpropanal (216 mg, 3 mmol) were added and the reaction was stirred to completion (TLC - R_f = 0.5 - EtOAc:Hexane - 1:1.22). After complete conversion of the olefin to the glycidic amide, the oxygen balloon was removed and a fresh lot of catalyst was added to the reaction mixture followed by *p*-toluidine (160 mg, 1.5 mmol). After stirring until complete consumption of the epoxide and formation of the product (TLC - R_f = 0.45 - EtOAc:Hexane - 3:2), washing of the unreacted material as described in the general procedure, with CCl_4 :hexane gave the β -phenylisoserine derived peptide **9d₁** in high purity (HPLC) and good yields (55 %), predominantly as the anti diastereomer.

^1H NMR, 300 MHz, CDCl_3 , δ 7.35 (m, 2H), 7.25 (m, 3H), 6.91 (d, J = 6 Hz, 2 H), 6.91 (m, 1H), 6.54 (d, J = 9 Hz, 2H), 6.10 (d, J = 9 Hz, 2H), 4.80 (dd, J = 12 Hz, 4.8 Hz, 1H), 4.74 (d, J = 5.1 Hz, 1 H), 4.46 - 4.37 (m, 2H), 3.71 (s, 3H), 3.66 - 3.54 (m, 2H), 2.19 (s, 3H), 2.06 - 1.95 (m, 2H), 1.70 - 1.65 (m, 2H), 1.56 - 1.51 (m, 1H), 1.46 (td, J = 14.4 Hz, 6 Hz, 2H), 0.95 (d, J = 6.3 Hz, 3H), 0.89 (dd, J = 6.3 Hz, 8 Hz, 3H); MS m/z : 495, 196, 211, 128; IR: ν_{max} : 3460(s), 3370, 3050, 3020, 2945, 1720, 1640 cm^{-1} .

Synthesis of methyl-N-(*p*-methoxyphenyl)- β -phenylisoserine-leucine-proline (9d₂)

To a solution of methyl-N-cinnamoyl-leucine-proline (558 mg, 1.5 mmol) in acetonitrile (7.5 mL), 2-methylpropanal (216 mg, 3 mmol) and PASCOS (~5mg) catalyst were added and the resulting solution was stirred under oxygen atmosphere for 12 h, after which a fresh lot of the catalyst and 2-methylpropanal (216 mg, 3 mmol) were added and the reaction was stirred to completion (TLC - R_f = 0.5 - EtOAc:Hexane - 1:1.22). After complete conversion of the olefin to the glycidic amide, the oxygen balloon was removed and a fresh lot of catalyst was added to the reaction mixture followed by *p*-anisidine (184 mg, 1.5 mmol). After stirring until complete consumption of the epoxide and formation of the product (TLC - R_f = 0.4 - EtOAc:Hexane - 3:2), washing of the unreacted material as described in the general procedure, with CCl_4 :hexane gave the β -phenylisoserine derived peptide **9d₂** in high purity (HPLC) and good yields (63 %), predominantly as the anti diastereomer.

^1H NMR, 300 MHz, CDCl_3 , δ 7.36 (m, 2H), 7.28 - 7.20 (m, 3H), 6.96 (d, J = 8.4 Hz, 1H), 6.69 (d, 8.8 Hz, 2 H), 6.60 (d, J = 8.8 Hz, 2 H), 4.82 (dd, J = 6.9 Hz, 3.6 Hz, 1 H),

5.08 (m, 1H), 4.56 (t, $J = 3.6$ Hz, 1H), 4.50 (dt, $J = 8.1$ Hz, 3.6 Hz, 1H), 3.78 (t, $J = 5.7$ Hz, 1H), 3.70 (s, 3H), 3.69 (s, 3H), 3.54 (m, 1H), 2.38 (m, 1H), 2.38 (m, 1H), 2.24 - 2.13 (m, 1H), 2.04 - 1.94 (m, 2H), 1.26 (dd, $J = 17.4$ Hz, 8 Hz, 2H), 0.91 (m, 1H), 0.80 (t, $J = 5.7$ Hz, 3H), 0.74 (t, $J = 5.7$ Hz, 3H); MS m/z : 511 (M^+), 299, 212, 242, 128; IR ν_{\max} 3400(s), 2980, 2950, 1740, 1660, 1640 cm^{-1}

Synthesis of methyl-N-(*p*-bromophenyl)- β -phenylisoserine-leucine-prolinate (**9d₃**)

To a solution of methyl-N-cinnamoyl-leucine-prolinate (558 mg, 1.5 mmol) in acetonitrile (7.5 mL), 2-methylpropanal (216 mg, 3 mmol) and PASCOS (~5mg) catalyst were added and the resulting solution was stirred under oxygen atmosphere for 12 h, after which a fresh lot of the catalyst and 2-methylpropanal (216 mg, 3 mmol) were added and the reaction was stirred to completion (TLC - $R_f = 0.5$ - EtOAc:Hexane - 1:1.22). After complete conversion of the olefin to the glycidic amide, the oxygen balloon was removed and a fresh lot of catalyst was added to the reaction mixture followed by *p*-bromoaniline (259 mg, 1.5 mmol). After stirring until complete consumption of the epoxide and formation of the product (TLC - $R_f = 0.5$ - EtOAc:Hexane - 3:2), washing of the unreacted material as described in the general procedure, with CCl_4 :hexane gave the β -phenylisoserine derived peptide **9d₃** in high purity (HPLC) and moderate yields (42 %), predominantly as the anti diastereomer.

^1H NMR, 400 MHz, CDCl_3 , δ 7.35 - 7.29 (m, 3H), 7.28 - 7.21 (m, 2H), 7.13 (d, $J = 8.4$ Hz, 2H), 6.97 (m, 1H), 6.47 (d, $J = 8.4$ Hz, 2H), 4.85 (dd, $J = 11.4$ Hz, 3 Hz, 1H), 4.76 (dd, $J = 14.4$ Hz, 4.5 Hz, 1H), 4.50 - 4.47 (m, 1H), 4.39 (dd, $J = 8.4$ Hz, 3 Hz, 1H), 3.78 - 3.71 (m, 1H), 3.69 (s, 3H), 3.51 - 3.49 (m, 1H), 2.22 - 2.14 (m, 1H), 2.09 - 1.92 (m, 3H), 1.53 - 1.47 (m, 1H), 1.45 - 1.43 (m, 1H), 1.23 (ddd, $J = 21$ Hz, 14.1 Hz, 7.2 Hz, 1H), 0.93 (d, $J = 6$ Hz, 3H), 0.89 (dd, $J = 6$ Hz, 3 Hz, 3H); MS m/z 560 (M^+), 299, 261, 128, 211; IR ν_{\max} 3210 (br), 2990, 2960, 1740, 1640, 1590 cm^{-1}

Synthesis of methyl-N-(*p*-methylphenyl)- β -phenylisoserine-proline-isoleucinate (**9e₁**)

To a solution of methyl-N-cinnamoyl-proline-isoleucinate (558 mg, 1.5 mmol) in acetonitrile (7.5 mL), 2-methylpropanal (216 mg, 3 mmol) and PASCOS (~5mg) catalyst were added and the resulting solution was stirred under oxygen atmosphere for 12 h, after which a fresh lot of the catalyst and 2-methylpropanal (216 mg, 3 mmol) were added and the reaction was stirred to completion (TLC - $R_f = 0.5$ - EtOAc:Hexane - 1:1). After complete conversion of the olefin to the glycidic amide, the oxygen balloon was removed and a fresh lot of catalyst was added to the reaction mixture followed by *p*-toluidine (160 mg, 1.5 mmol). After stirring until complete consumption of the epoxide and formation of the product (TLC - $R_f = 0.4$ - EtOAc:Hexane - 3:2), washing of the unreacted material as described in the general procedure, with CCl_4 :hexane gave the β -phenylisoserine derived peptide **9e₁** in high purity (HPLC) and moderate yields (48 %), predominantly as the anti diastereomer.

^1H NMR, 300 MHz, CDCl_3 , δ 7.34 - 7.26 (m, 6H), 6.89 (dd, $J = 9$ Hz & 7.5 Hz, 2H), 6.48 (dd, $J = 13$ Hz & 9 Hz, 2H), 4.79 (d, $J = 5.7$ Hz, 1H), 4.63 (d, $J = 5.7$ Hz, 1H), 4.53 - 4.48 (m, 2H), 3.74 (s, 3H), 3.67 (t, $J = 7.8$ Hz, 1H), 3.40 - 3.28 (m, 1H), 2.53 - 2.50 (m, 1H), 2.218 (m, 1H), 2.17 (s, 3H), 2.05 - 1.88 (m, 2H), 1.57 - 1.53 (m, 2H), 1.38 - 1.36 (m,

1H), 1.24 - 1.15 (m, 1H), 0.89 (t, $J = 7.3$ Hz, 3H), 0.87 (d, $J = 6.84$ Hz, 3H); MS m/z 495 (M^+), 464, 436, 299, 196, 91; IR ν_{\max} 3300, 3030, 2990, 2880, 1775, 1660, 1600 cm^{-1}

Synthesis of methyl-N-(*p*-methoxyphenyl)- β -phenylisoserine-proline-isoleucinate (9e₂)

To a solution of methyl-N-cinnamoyl-proline-isoleucinate (558 mg, 1.5 mmol) in acetonitrile (7.5 mL), 2-methylpropanal (216 mg, 3 mmol) and PASCOS (~5mg) catalyst were added and the resulting solution was stirred under oxygen atmosphere for 12 h, after which a fresh lot of the catalyst and 2-methylpropanal (216 mg, 3 mmol) were added and the reaction was stirred to completion (TLC - $R_f = 0.5$ - EtOAc:Hexane - 1:1). After complete conversion of the olefin to the glycidic amide, the oxygen balloon was removed and a fresh lot of catalyst was added to the reaction mixture followed by *p*-anisidine (184 mg, 1.5 mmol). After stirring until complete consumption of the epoxide and formation of the product (TLC - $R_f = 0.5$ - EtOAc:Hexane - 7:3), washing of the unreacted material as described in the general procedure, with CCl_4 :hexane gave the β -phenylisoserine derived peptide 9e₂ in high purity (HPLC) and good yields (54 %), predominantly as the anti diastereomer.

^1H NMR, CDCl_3 , 300 MHz, δ 7.33 (d, $J = 7.8$ Hz, 1H), 7.34 - 7.23 (m, 5H), 6.69 (d, $J = 9$ Hz, 2H), 6.55 (d, $J = 9$ Hz, 2H), 4.77 (d, $J = 4.4$ Hz, 1H), 4.64 (d, $J = 4.4$ Hz, 1H), 4.47 (t, $J = 8.6$ Hz, 1H), 4.46 (d, $J = 8.6$ Hz, 1H), 3.73 (s, 3H), 3.69 (s, 3H), 3.41 (dd, $J = 9.04$ Hz & 2.7 Hz, 1H), 2.52 (dd, $J = 9.5$ Hz & 2.4 Hz, 1H), 2.05 (m, 1H), 1.90 - 1.85 (m, 2H), 1.57 - 1.53 (m, 2H), 1.38 - 1.36 (m, 1H) 1.27 - 1.25 (m, 1H), 1.24 - 1.15 (m, 1H), 0.89 (t, $J = 7.3$ Hz, 3H), 0.87 (d, $J = 6.8$ Hz, 3H); MS m/z 511, 480, 452, 299, 243, 212, 107; IR ν_{\max} 3390, 3050, 2880, 1725, 1660, 1600 cm^{-1}

Synthesis of methyl-N-(*p*-bromophenyl)- β -phenylisoserine-proline-isoleucinate (9e₃)

To a solution of methyl-N-cinnamoyl-proline-isoleucinate (558 mg, 1.5 mmol) in acetonitrile (7.5 mL), 2-methylpropanal (216 mg, 3 mmol) and PASCOS (~5mg) catalyst were added and the resulting solution was stirred under oxygen atmosphere for 12 h, after which a fresh lot of the catalyst and 2-methylpropanal (216 mg, 3 mmol) were added and the reaction was stirred to completion (TLC - $R_f = 0.5$ - EtOAc:Hexane - 1:1). After complete conversion of the olefin to the glycidic amide, the oxygen balloon was removed and a fresh lot of catalyst was added to the reaction mixture followed by *p*-bromoaniline (259 mg, 1.5 mmol). After stirring until complete consumption of the epoxide and formation of the product (TLC - $R_f = 0.5$ - EtOAc:Hexane - 3:2), washing of the unreacted material as described in the general procedure, with CCl_4 :hexane gave the β -phenylisoserine derived peptide 9e₃ in high purity (HPLC) and moderate yields (45 %), predominantly as the anti diastereomer.

^1H NMR CDCl_3 , 300 MHz, δ 7.32 - 7.25 (m, 5H), 7.23 (d, $J = 7.2$ Hz, 1H), 6.97 (d, $J = 8$ Hz, 2H), 6.67 (d, $J = 8$ Hz, 2H), 4.65 (d, $J = 5.6$ Hz, 1H), 4.59 (d, $J = 5.6$ Hz, 1H), 4.49 (d, $J = 9.8$ Hz, 1H), 4.42 (dd, $J = 6.1$ Hz & 3 Hz, 1H), 3.69 (s, 3H), 3.65 - 3.49 (m, 2H), 2.49 - 2.41 (m, 1H), 2.32 - 2.16 (m, 2H), 2.12 - 2.09 (m, 1H), 1.52 - 1.38 (m, 2H), 1.15 - 1.02 (m, 1H), 0.98 (dd, $J = 9.2$ Hz & 5.2 Hz, 3H), 0.88 (d, $J = 6.7$ Hz, 3H); IR ν_{\max} 3300, 2800, 1725, 1615 cm^{-1}

Synthesis of methyl-N-(*p*-methylphenyl)- β -phenylisoserine-proline-aspartate (**9f₁**)

To a solution of methyl-N-cinnamoyl-proline-aspartate (582 mg, 1.5 mmol) in acetonitrile (7.5 mL), 2-methylpropanal (216 mg, 3 mmol) and PASCOS (~5mg) catalyst were added and the resulting solution was stirred under oxygen atmosphere for 12 h, after which a fresh lot of the catalyst and 2-methylpropanal (216 mg, 3 mmol) were added and the reaction was stirred to completion (TLC - R_f = 0.45 - EtOAc:Hexane - 1:1). After complete conversion of the olefin to the glycidic amide, the oxygen balloon was removed and a fresh lot of catalyst was added to the reaction mixture followed by *p*-toluidine (160 mg, 1.5 mmol). After stirring until complete consumption of the epoxide and formation of the product (TLC - R_f = 0.4 - EtOAc:Hexane - 3:2), washing of the unreacted material as described in the general procedure, with CCl_4 :hexane gave the β -phenylisoserine derived peptide **9f₁** in high purity (HPLC) and good yields (56 %), predominantly as the anti diastereomer.

^1H NMR, 400 MHz, CDCl_3 , δ 7.54 (dd, J = 7.52 Hz & 4.1 Hz, 1H), 7.43 - 7.19 (m, 5H), 6.88 (d, J = 8.6 Hz, 2H), 6.51 (d, J = 8.6 Hz, 2H), 4.75 (td, J = 7.6 Hz & 4.6 Hz, 1H), 4.71 (d, J = 7.9 Hz, 1H), 4.65 - 4.62 (m, 1H), 4.45 (dd, J = 7.6 Hz & 4.2 Hz, 1H), 3.77 (s, 3H), 3.75 - 3.64 (m, 2H), 3.63 (s, 3H), 2.98 (dd, J = 8.6 Hz & 5.4 Hz, 1H), 2.94 (dd, J = 8.6 Hz & 5.4 Hz, 1H), 2.23 - 2.19 (m, 1H), 2.16 (s, 3H), 2.07 - 1.86 (m, 2H), 1.81 - 1.77 (m, 1H); IR ν_{max} 3300(br), 3020, 2080, 1775, 1650 cm^{-1}

Synthesis of methyl-N-(*p*-methoxyphenyl)- β -phenylisoserine-proline-aspartate (**9f₂**)

To a solution of methyl-N-cinnamoyl-proline-aspartate (582 mg, 1.5 mmol) in acetonitrile (7.5 mL), 2-methylpropanal (216 mg, 3 mmol) and PASCOS (~5mg) catalyst were added and the resulting solution was stirred under oxygen atmosphere for 12 h, after which a fresh lot of the catalyst and 2-methylpropanal (216 mg, 3 mmol) were added and the reaction was stirred to completion (TLC - R_f = 0.45 - EtOAc:Hexane - 1:1). After complete conversion of the olefin to the glycidic amide, the oxygen balloon was removed and a fresh lot of catalyst was added to the reaction mixture followed by *p*-anisidine (184 mg, 1.5 mmol). After stirring until complete consumption of the epoxide and formation of the product (TLC - R_f = 0.45 - EtOAc:Hexane - 1:1), washing of the unreacted material as described in the general procedure, with CCl_4 :hexane gave the β -phenylisoserine derived peptide **9f₂** in high purity (HPLC) and good yields (60 %), predominantly as the anti diastereomer.

^1H NMR, 400 MHz, CDCl_3 , δ 7.36 (d, J = 8.3 Hz, 1H), 7.29 - 7.11 (m, 5H), 6.46 (d, J = 6.8 Hz, 2H), 6.42 (d, J = 6.8 Hz, 2H), 4.76 (dd, J = 13.4 Hz & 5.1 Hz, 1H), 4.65 (d, J = 4.4 Hz, 1H), 4.56 (dd, J = 8.3 Hz & 3.6 Hz, 1H), 4.38 (dd, J = 8 Hz & 5.1 Hz, 1H), 3.71 - 3.69 (m, 1H), 3.66 (s, 3H), 3.57 (s, 3H), 3.53 (s, 3H), 3.52 - 3.49 (m, 1H), 2.86 (dd, J = 6.8 Hz & 3.6 Hz, 1H), 2.81 (d, J = 4.9 Hz, 1H), 2.25 - 2.18 (m, 1H), 2.11 - 2.06 (m, 1H), 1.96 - 1.87 (m, 1H), 1.83 - 1.77 (m, 1H); IR ν_{max} 3400(br), 3320(s), 2200, 2080, 1710, 1660 cm^{-1}

Synthesis of Aziridine containing tripeptide (**10**)

To a solution of the tripeptide methyl-N-(*p*-bromophenyl)- β -phenylisoserine-leucine-prolinate (**9d₃**) (280 mg, 0.5 mmol) in CH_3CN (8 mL) was added triethylamine (0.08 mL, 0.55 mmol), DEAD (87 mg, 0.5 mmol) and PPh_3 (132 mg, 0.5 mmol) and the reaction

mixture was allowed to stir over night. The solvent was removed and the resulting residue was purified by subjecting to flash column chromatography (Silica gel-EtOAc:Hexane - 3:2) to yield the aziridine containing tripeptide **10** in good yields (63 %).

^1H NMR, 400 MHz, CDCl_3 , δ 7.29-7.19 (m, 7H), 7.12-7.07 (m, 1H), 6.66 (d, $J = 8.8\text{Hz}$, 1H), 6.61 (d, $J = 8.8\text{Hz}$, 1H), 4.93 (ddd, $J = 21.2, 8.4$ and 5.2Hz , 0.5H), 4.82 (ddd, $J = 19.2, 10.4$ and 5.2Hz , 0.5H), 4.51 (dd, $J = 8.8$ and 2.8Hz , 1H), 3.76 (s, 3H), 3.74 (d, $J = 2.8\text{Hz}$, 1H), 3.62-3.50 (m, 2H), 3.28 (d, $J = 2.8\text{Hz}$, 1H), 2.06-1.97 (m, 3H), 1.56-1.53 (m, 1H), 1.30-1.15 (m, 3H), 0.97 (dd, $J = 10.6$ and 3Hz), 0.90 (t, $J = 6\text{Hz}$, 3H)

Synthesis of methyl-((β -methoxy)-N-(*p*-bromophenyl)phenylalanine-leucine-prolinate (**11**))

To a solution of the aziridine containing peptide (**10**) (135 mg, 0.25 mmol) in methanol (5 mL) was added tosylchloride (catalytic) and the reaction mixture was stirred for 4 h. The solvent was removed under vacuum to yield a residue, which was taken in EtOAc (10 mL) and washed with saturated solution of NaHCO_3 (2X3 mL), water (2X3 mL) and brine (1X3 mL). The organic phase was separated, dried (Na_2SO_4) and concentrated in vacuum to yield a residue which was subjected to column chromatography (Silica gel-EtOAc:Hexane - 3:2) to yield the corresponding phenylalanine derivative in good yields (78 %).

^1H NMR, 400 MHz, CDCl_3 , δ 7.35-7.32 (m, 4H), 7.30-7.23 (m, 1H), 7.19 (d, $J = 8.8\text{Hz}$, 2H), 7.08 (d, $J = 8.8\text{Hz}$, 1H), 6.40 (d, $J = 8.8\text{Hz}$, 2H), 5.01 (t, $J = 4.8\text{Hz}$, 1H), 4.80 (dt, $J = 8.8$ and 5.2Hz , 1H), 4.47 (dd, $J = 8.8$ and 4.4Hz , 1H), 4.30 (d, $J = 5.6\text{Hz}$, 1H), 3.97 (t, $J = 6\text{Hz}$, 1H), 3.80 (m, 1H), 3.70 (s, 3H), 3.54-3.44 (m, 1H), 2.22-2.13 (m, 1H), 2.11-1.91 (m, 3H), 1.75 bs, 1H), 1.50-1.38 (m, 1H), 1.35 (m, 2H), 0.88 (d, $J = 5.6\text{Hz}$, 3H), 0.79 (dd, $J = 6$ and 3.6Hz , 3H); MS m/z : 560 (M^+), 501, 453, 297, 107; IR ν_{max} 3400 (br), 3300 (s), 3030, 2890, 1775, 1715, 1660, 1605

Synthesis of methyl-N-(*m*-hydroxyphenyl)- β -phenylisoserine-proline-leucinate (**12a**)

To a solution of methyl-N-cinnamoyl-proline-leucinate (558 mg, 1.5 mmol) in acetonitrile (7.5 mL), 2-methylpropanal (216 mg, 3 mmol) and PASCOS (~5mg) catalyst were added and the resulting solution was stirred under oxygen atmosphere for 12 h, after which a fresh lot of the catalyst and 2-methylpropanal (216 mg, 3 mmol) were added and the reaction was stirred to completion (TLC - $R_f = 0.5$ - EtOAc:Hexane - 1:1). After complete conversion of the olefin to the glycidic amide, the oxygen balloon was removed and a fresh lot of catalyst was added to the reaction mixture followed by *m*-aminophenol (164 mg, 1.5 mmol). After stirring until complete consumption of the epoxide and formation of the product (TLC - $R_f = 0.3$ - EtOAc:Hexane - 3:2), washing of the unreacted material as described in the general procedure, with CCl_4 :hexane gave the β -phenylisoserine derived peptide **12a** in high purity (HPLC) and good yields (56 %), predominantly as the anti diastereomer.

^1H NMR, 300 MHz, CDCl_3 , δ 7.37 - 7.19 (m, 6H), 7.12 (d, $J = 7.8\text{Hz}$, 1H), 6.87 (m, 3H), 4.78 (d, $J = 6.8\text{Hz}$, 1H), 4.71 - 4.56 (m, 2H), 4.32 (t, $J = 5.6\text{Hz}$, 1H), 3.79 - 3.72 (m, 1H), 3.70 (s, 3H), 3.67 - 3.58 (m, 1H), 2.47 - 2.40 (m, 1H), 2.38 - 2.32 (m, 1H), 2.24

- 2.12 (m, 2H), 1.56 - 1.38 (m, 2H), 1.15 - 1.08 (m, 1H), 0.96 (d, $J = 6.5$ Hz, 3H), 0.88 (d, $J = 6.5$ Hz, 3H); IR ν_{max} 3400(s), 3020, 2900, 1715, 1600, 1480 cm^{-1}

Synthesis of methyl-N-(*m*-hydroxyphenyl)- β -phenylisoserine-proline-aspartate (12b)

To a solution of methyl-N-cinnamoyl-proline-aspartate (582 mg, 1.5 mmol) in acetonitrile (7.5 mL), 2-methylpropanal (216 mg, 3 mmol) and PASCOS (~5mg) catalyst were added and the resulting solution was stirred under oxygen atmosphere for 12 h, after which a fresh lot of the catalyst and 2-methylpropanal (216 mg, 3 mmol) were added and the reaction was stirred to completion (TLC - $R_f = 0.45$ - EtOAc:Hexane - 1:1). After complete conversion of the olefin to the glycidic amide, the oxygen balloon was removed and a fresh lot of catalyst was added to the reaction mixture followed by *m*-aminophenol (164 mg, 1.5 mmol). After stirring until complete consumption of the epoxide and formation of the product (TLC - $R_f = 0.33$ - EtOAc:Hexane - 7:3), washing of the unreacted material as described in the general procedure, with EtOAc:CCl₄:hexane gave the β -phenylisoserine derived peptide **12b** in high purity (HPLC) and good yields (60 %), predominantly as the anti diastereomer.

¹H NMR, 400 MHz, CDCl₃, δ 7.84 (d, $J = 8.08$ Hz, 1H), 7.75 - 7.26 (m, 8H), 6.22 - 6.05 (m, 1H), 4.85 (d, $J = 4.9$ Hz, 1H), 4.76 (d, $J = 4.4$ Hz, 1H), 4.71 (dd, $J = 16.6$ and 7.2 Hz, 1H), 4.55 (bs, 1H), 3.74 (s, 3H), 3.70 (s, 3H), 3.66 - 3.64 (m, 2H), 2.94 - 2.80 (m, 2H), 2.36 - 1.91 (m, 4H); MS m/z (M^+ absent), 316, 259, 197.

Synthesis of methyl-N-(*m*-hydroxyphenyl)- β -phenylisoserine-proline-phenylalaninate (12c)

To a solution of methyl-N-cinnamoyl-proline-phenylalaninate (610 mg, 1.5 mmol) in acetonitrile (7.5 mL), 2-methylpropanal (216 mg, 3 mmol) and PASCOS (~5mg) catalyst were added and the resulting solution was stirred under oxygen atmosphere for 12 h, after which a fresh lot of the catalyst and 2-methylpropanal (216 mg, 3 mmol) were added and the reaction was stirred to completion (TLC - $R_f = 0.45$ - EtOAc:Hexane - 1:1). After complete conversion of the olefin to the glycidic amide, the oxygen balloon was removed and a fresh lot of catalyst was added to the reaction mixture followed by *m*-aminophenol (164 mg, 1.5 mmol). After stirring until complete consumption of the epoxide and formation of the product (TLC - $R_f = 0.3$ - EtOAc:Hexane - 7:3), washing of the unreacted material as described in the general procedure, with EtOAc:CCl₄:hexane gave the β -phenylisoserine derived peptide **12c** in high purity (HPLC) and good yields (54 %), predominantly as the anti diastereomer.

¹H NMR, CDCl₃, 300 MHz, δ 7.34 (d, $J = 7.2$ Hz, 1H), 7.30 - 7.22 (m, 6H), 7.10 (dd, $J = 14.7$ Hz & 6 Hz, 2H), 6.94 - 6.87 (m, 1H), 6.71 (d, $J = 7.8$ Hz, 1H), 6.16 - 6.02 (m, 3H), 4.81 - 4.74 (m, 2H), 4.67 - 4.58 (m, 1H), 4.40 - 4.32 (m, 1H), 3.66 (s, 3H), 3.35 - 3.32 (m, 1H), 3.17 (dt, $J = 15.3$ Hz & 6.3 Hz, 1H), 2.98 - 2.91 (m, 2H), 2.02 (dd, $J = 11.4$ Hz & 5.4 Hz, 2H), 1.84 (qd, $J = 25.2$ Hz, 6.9 Hz, 2H), 1.71 (dd, $J = 12$ Hz & 6 Hz, 2H); MS m/z : 531(M^+), 333, 301, 198

Synthesis of methyl-N-(*m*-hydroxyphenyl)- β -phenylisoserine-leucine-prolinate (13a)

To a solution of methyl-N-cinnamoyl-leucine-prolinate (558 gm, 1.5 mmol) in acetonitrile (7.5 mL), 2-methylpropanal (216 mg, 3 mmol) and PASCOS (~5mg) catalyst

were added and the resulting solution was stirred under oxygen atmosphere for 12 h, after which a fresh lot of the catalyst and 2-methylpropanal (216 mg, 3 mmol) were added and the reaction was stirred to completion (TLC - R_f = 0.5 - EtOAc:Hexane - 1:1.22). After complete conversion of the olefin to the glycidic amide, the oxygen balloon was removed and a fresh lot of catalyst was added to the reaction mixture followed by *m*-aminophenol (164 mg, 1.5 mmol). After stirring until complete consumption of the epoxide and formation of the product (TLC - R_f = 0.5 - EtOAc:Hexane - 7:3), washing of the unreacted material as described in the general procedure, with EtOAc:CCl₄:hexane gave the β -phenylisoserine derived peptide **13a** in high purity (HPLC) and good yields (53 %), predominantly as the anti diastereomer.

¹H NMR, 300MHz, d 7.41 - 7.38 (m, 2H), 7.27 - 7.21 (m, 3H), 6.95 (t, *J* = 8.1 Hz, 1H), 6.25 - 6.06 (m, 3H), 4.79 (d, *J* = 2.7 Hz, 1H), 4.62 (d, *J* = 2.7 Hz, 1H), 4.59 - 4.57 (m, 1H), 4.44 (dd, *J* = 11.7 Hz, 3.6 Hz, 1H), 2.07 - 1.96 (m, 1H), 1.73 (m, 3H), 1.33 (t, *J* = 7.2 Hz, 1H), 1.27 (t, *J* = 7.2 Hz, 1H), 0.94 - 0.89 (m, 1H), 0.77 - 0.69 (m, 6H); MS *m/z*: 497, 198, 299, 128; IR ν_{\max} 3380(s), 3060, 3030, 2960, 2880, 1740, 1640 cm⁻¹

Synthesis of methyl-N-(*m*-hydroxyphenyl)- β -phenylisoserine-phenylalanine-prolinate (**13b**)

To a solution of methyl-N-cinnamoyl-phenylalanine-prolinate (610 mg, 1.5 mmol) in acetonitrile (7.5 mL), 2-methylpropanal (216 mg, 3 mmol) and PASCOS (~5mg) catalyst were added and the resulting solution was stirred under oxygen atmosphere for 12 h, after which a fresh lot of the catalyst and 2-methylpropanal (216 mg, 3 mmol) were added and the reaction was stirred to completion (TLC - R_f = 0.4 - EtOAc:Hexane - 3:2). After complete conversion of the olefin to the glycidic amide, the oxygen balloon was removed and a fresh lot of catalyst was added to the reaction mixture followed by *m*-aminophenol (164 mg, 1.5 mmol). After stirring until complete consumption of the epoxide and formation of the product (TLC - R_f = 0.25 - EtOAc:Hexane - 7:3), washing of the unreacted material as described in the general procedure, with EtOAc:CCl₄:hexane gave the β -phenylisoserine derived peptide **13b** in high purity (HPLC) and good yields (52 %), predominantly as the anti diastereomer.

¹H NMR, 300 MHz, CDCl₃, δ 7.61 - 6.77 (m, 7H), 6.19 (d, *J* = 10.8 Hz, 1H), 6.12 (bs, 1H), 4.71 (dd, *J* = 9.2 Hz & 6 Hz, 1H), 4.44 (d, *J* = 5.6 Hz, 1H), 4.38 (d, *J* = 5.6 Hz, 1H), 4.28 (dd, *J* = 10.4 Hz & 6 Hz, 1H), 3.64 (s, 3H), 3.40 - 3.37 (m, 1H), 3.09 - 3.05 (m, 2H), 2.93 - 2.88 (m, 1H), 2.59 - 2.57 (m, 1H), 2.11 - 2.06 (m, 1H), 1.90 - 1.78 (m, 2H); MS *m/z* (*M*⁺absent) 334, 304, 232(100%); IR ν_{\max} 3360(br), 3050, 2950, 1715, 1610 cm⁻¹

Synthesis of methyl-N-(*m*-hydroxyphenyl)- β -phenylisoserine-leucine-(4-*trans*-acetoxy)prolinate (**13c**)

To a solution of methyl-N-cinnamoyl-leucine-(4-*trans*-acetoxy)prolinate (675 mg, 1.5 mmol) in acetonitrile (7.5 mL), 2-methylpropanal (216 mg, 3 mmol) and PASCOS (~5mg) catalyst were added and the resulting solution was stirred under oxygen atmosphere for 12 h, after which a fresh lot of the catalyst and 2-methylpropanal (216 mg, 3 mmol) were added and the reaction was stirred to completion (TLC - R_f = 0.5 - EtOAc:Hexane - 1.1). After complete conversion of the olefin to the glycidic amide, the oxygen balloon was removed and a fresh lot of catalyst was added to the reaction mixture followed by *m*-aminophenol (164 mg, 1.5 mmol). After stirring until complete consumption of the epoxide and formation of the product (TLC - R_f = 0.3 -

EtOAc:Hexane - 7:3), washing of the unreacted material as described in the general procedure, with EtOAc:CCl₄:hexane gave the β -phenylisoserine derived peptide **13c** in high purity (HPLC) and good yields (55 %), predominantly as the anti diastereomer.

¹H NMR, 300 MHz, CDCl₃, δ 7.42 - 7.21 (m, 5H), 7.12 (d, *J* = 8.4 Hz, 1H), 6.94 (t, *J* = 8.4 Hz, 1H), 6.19 - 6.14 (m, 3H), 5.37 - 5.33 (m, 1H), 4.78 (d, *J* = 4.8 Hz, 1H), 4.71 - 4.61 (m, 1H), 4.56 (t, *J* = 4.8 Hz, 1H), 4.71 - 4.61 (m, 1H), 4.56 (t, *J* = 8.1 Hz, 1H), 4.35 (dd, *J* = 9.9 Hz & 6.9 Hz, 1H), 3.98 - 3.80 (m, 1H), 3.73 (s, 3H), 3.52 - 3.50 (m, 1H), 2.45 - 2.30 (m, 1H), 2.19 - 2.08 (m, 1H), 2.07 (s, 3H), 1.57 - 1.50 (m, 1H), 1.46 (t, *J* = 6 Hz, 2H), 0.95 - 0.84 (m, 6H); MS *m/z*: 357, 198, 187; IR ν_{\max} 3300(br), 3030, 2920, 1675 cm⁻¹

Synthesis of methyl-N-(*m*-hydroxyphenyl)- β -phenylisoserine-valine-prolinate (**13d**)

To a solution of methyl-N-cinnamoyl-valine-prolinate (537 mg, 1.5 mmol) in acetonitrile (7.5 mL), 2-methylpropanal (216 mg, 3 mmol) and PASCOS (~5mg) catalyst were added and the resulting solution was stirred under oxygen atmosphere for 12 h, after which a fresh lot of the catalyst and 2-methylpropanal (216 mg, 3 mmol) were added and the reaction was stirred to completion (TLC - *R_f* = 0.5 - EtOAc:Hexane - 1:1.2). After complete conversion of the olefin to the glycidic amide, the oxygen balloon was removed and a fresh lot of catalyst was added to the reaction mixture followed by *m*-aminophenol (164 mg, 1.5 mmol). After stirring until complete consumption of the epoxide and formation of the product (TLC - *R_f* = 0.4 - EtOAc:Hexane - 7:3), washing of the unreacted material as described in the general procedure, with EtOAc:CCl₄:hexane gave the β -phenylisoserine derived peptide **13d** in high purity (HPLC) and moderate yields (47 %), predominantly as the anti diastereomer.

¹H NMR, 300 MHz, CDCl₃, δ 7.36 - 7.30 (m, 1H), 7.28 - 7.22 (m, 3H), 7.05 (d, *J* = 8.7 Hz, 1H), 6.93 (t, *J* = 7.8 Hz, 1H), 6.18 (d, *J* = 8.1 Hz, 1H), 6.15 - 6.13 (m, 2H), 4.92 - 4.85 (m, 1H), 4.78 (dd, *J* = 9.9 Hz, 4.5 Hz, 1H), 4.48 (d, *J* = 4.5 Hz, 1H), 4.44 - 4.40 (m, 1H), 3.67 (s, 3H), 3.59 - 3.41 (m, 2H), 2.18 - 2.12 (m, 1H), 2.09 - 2.03 (m, 2H), 1.86 - 1.73 (m, 2H), 1.27 (t, *J* = 7.2 Hz, 1H), 0.92 (dd, *J* = 6 Hz & 2.1 Hz, 3H); MS *m/z* 497(M⁺), 299, 198, 128

Synthesis of methyl-N-(*m*-hydroxyphenyl)- β -phenylisoserine-valine-(4-*trans*-hydroxy)prolinate (**13e**)

To a solution of methyl-N-cinnamoyl-valine-(4-*trans*-hydroxy)prolinate (588 mg, 1.5 mmol) in acetonitrile (7.5 mL), 2-methylpropanal (216 mg, 3 mmol) and PASCOS (~5mg) catalyst were added and the resulting solution was stirred under oxygen atmosphere for 12 h, after which a fresh lot of the catalyst and 2-methylpropanal (216 mg, 3 mmol) were added and the reaction was stirred to completion (TLC - *R_f* = 0.4 - EtOAc:Hexane - 7:3). After complete conversion of the olefin to the glycidic amide, the oxygen balloon was removed and a fresh lot of catalyst was added to the reaction mixture followed by *m*-aminophenol (164 mg, 1.5 mmol). After stirring until complete consumption of the epoxide and formation of the product (TLC - *R_f* = 0.2 - EtOAc:Hexane - 8:2), washing of the unreacted material as described in the general procedure, with EtOAc:CCl₄:hexane gave the β -phenylisoserine derived peptide **13e** in poor purity (HPLC) and very moderate yields (28 %).

^1H NMR, 300 MHz, CDCl_3 , δ 7.53-7.00 (m, 6H), 6.92(d, J = 8Hz, 1H), 6.10 (d, J = 8Hz, 3H), 5.20 (dd, J = 6.5Hz & J = 5.6Hz, 1H), 4.71 (d, J = 6.6Hz, 1H), 4.56 (d, J = 7.2Hz, 1H), 4.40 (d, J = 9.6Hz, 1H), 4.28 - 4.19 (m, 1H), 3.96 - 3.78 (m, 1H), 3.66 (s, 3H), 3.42 (t, J = 5.6Hz, 1H), 1.22 (dd, J = 12.8Hz & J = 8Hz, 2H), 2.03 - 1.96 (m, 1H), 0.86 (d, J = 5.8Hz, 6H)

Synthesis of methyl-N-(*m*-hydroxyphenyl)- β -phenylisoserine-valine-(4-*trans*-acetoxy)prolinate (13f)

To a solution of methyl-N-cinnamoyl-valine-(4-*trans*-acetoxy)ptolinate (624 mg, 1.5 mmol) in acetonitrile (7.5 mL), 2-methylpropanal (216 mg, 3 mmol) and PASCOS (~5mg) catalyst were added and the resulting solution was stirred under oxygen atmosphere for 12 h, after which a fresh lot of the catalyst and 2-methylpropanal (216 mg, 3 mmol) were added and the reaction was stirred to completion (TLC - R_f = 0.45 - EtOAc:Hexane - 1:1). After complete conversion of the olefin to the glycidic amide, the oxygen balloon was removed and a fresh lot of catalyst was added to the reaction mixture followed by *m*-aminophenol (164 mg, 1.5 mmol). After stirring until complete consumption of the epoxide and formation of the product (TLC - R_f = 0.4 - EtOAc:Hexane - 7:3), washing of the unreacted material as described in the general procedure, with EtOAc: CCl_4 :hexane gave the β -phenylisoserine derived peptide 13f in high purity (HPLC) and good yields (50 %), predominantly as the anti diastereomer.

^1H NMR, 300 MHz, CDCl_3 , δ 7.44 - 7.00 (m, 6H), 6.84 (d, J = 10.4Hz, 1H), 6.08 (d, J = 7.2Hz, 3H), 5.34 - 5.03 (m, 1H), 4.72 (dd, J = 5.6Hz & 5.6Hz, 1H), 4.45 (d, J = 5.6Hz, 1H), 4.31 (dd, J = 6.6Hz & 4Hz, 1H), 1.03 (dd, J = 9.6Hz & 2.4Hz, 1H), 3.92 - 3.73 (m, 2H), 3.62 (s, 3H), 2.11 (dd, J = 12.8Hz & 4.8Hz, 2H), 1.95 (s, 3H), 1.28 - 1.10 (m, 1H), 0.81 (dd, J = 14.4Hz & 9.6Hz, 6H)

Synthesis of methyl-N,N-(phenyl(*m*-hydroxy-*p*-methoxybenzyl))- β -phenylisoserine- proline -isoleucinate (14)

To a solution of methyl-N-cinnamoyl-proline-leucinate (558 mg, 1.5 mmol) in acetonitrile (7.5 mL), 2-methylpropanal (216 mg, 3 mmol) and PASCOS (~5mg) catalyst were added and the resulting solution was stirred under oxygen atmosphere for 12 h, after which a fresh lot of the catalyst and 2-methylpropanal (216 mg, 3 mmol) were added and the reaction was stirred to completion (TLC - R_f = 0.5 - EtOAc:Hexane - 1:1). After complete conversion of the olefin to the glycidic amide, the oxygen balloon was removed and a fresh lot of catalyst was added to the reaction mixture followed by the aromatic secondary amine N,N-phenyl-(*m*-hydroxy-*p*-methoxybenzyl)amine 8e, (343 mg, 1.5 mmol). After stirring until complete consumption of the epoxide and formation of the product (TLC - R_f = 0.2 - EtOAc:Hexane - 7:3), washing of the unreacted material as described in the general procedure, with EtOAc: CCl_4 :hexane gave the β -phenylisoserine derived peptide 14 in high purity (HPLC) and good yields (50 %), predominantly as the anti diastereomer.

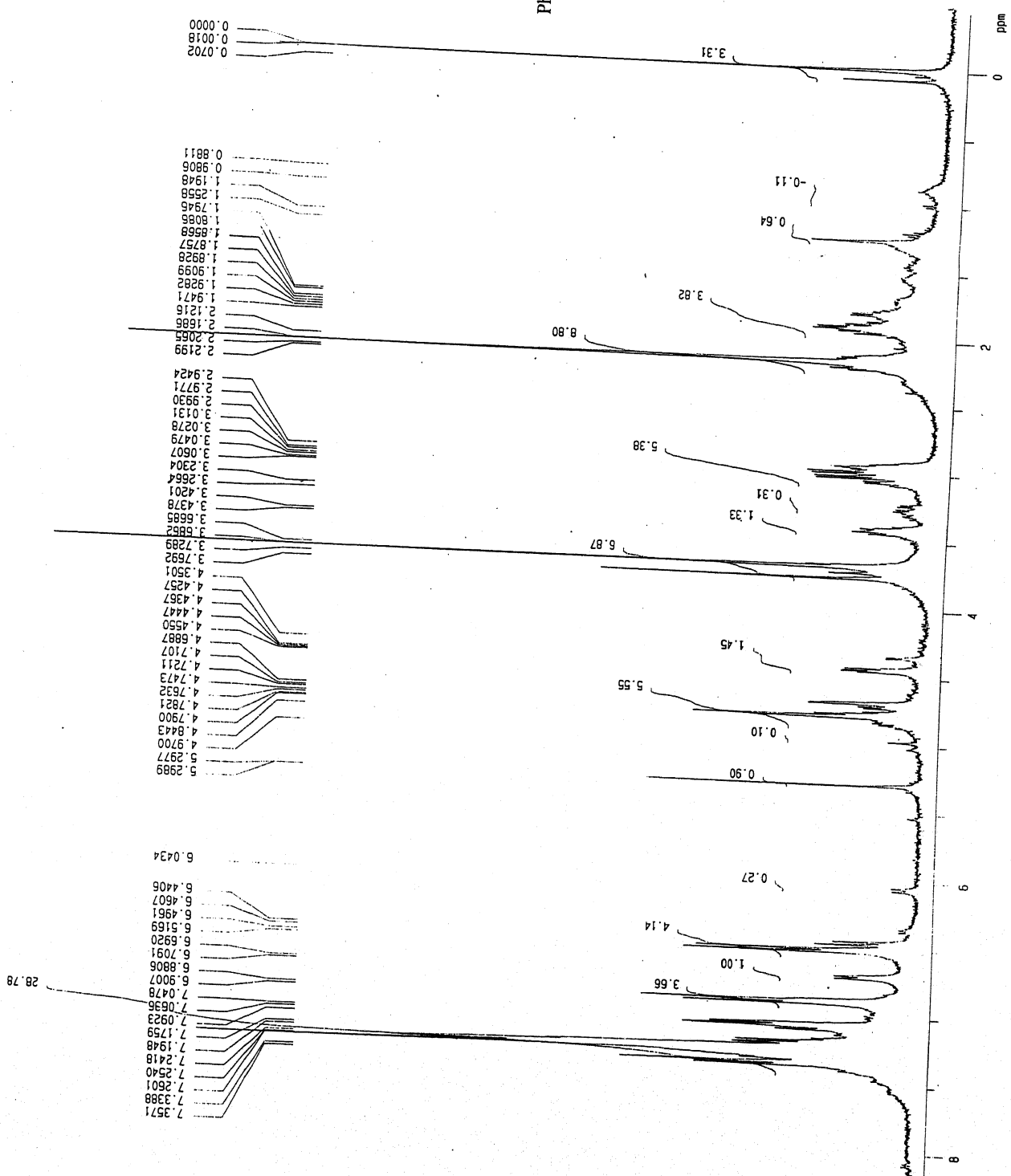
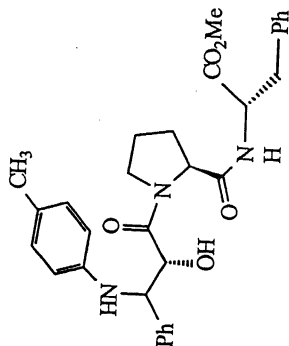
^1H NMR, 300 MHz, CDCl_3 , δ 7.30 (m, 1H), 7.25 - 7.21 (m, 5H), 6.83 (d, J = 8.8 Hz, 2H), 6.70 (d, J = 8.8 Hz, 2H), 5.88 - 5.80 (m, 2H), 5.45 (d, J = 16 Hz, 1H), 5.23 (d, J = 6 Hz, 1H), 5.20 (d, J = 16 Hz, 1H), 4.90 (d, J = 4.1 Hz, 1H), 4.86 (d, J = 4.1 Hz, 1H), 4.62 (dd, J = 12.2 Hz & 4.8 Hz, 2H), 4.55-4.53 (m, 2H), 4.25 (d, J = 12 Hz, 1H), 3.85 (s, 1H), 3.69 (s, 3H), 3.60 (d, J = 6.2 Hz, 1H), 3.52 (d, J = 6.2 Hz, 1H), 2.35 - 2.31 (m, 1H), 2.24-2.12

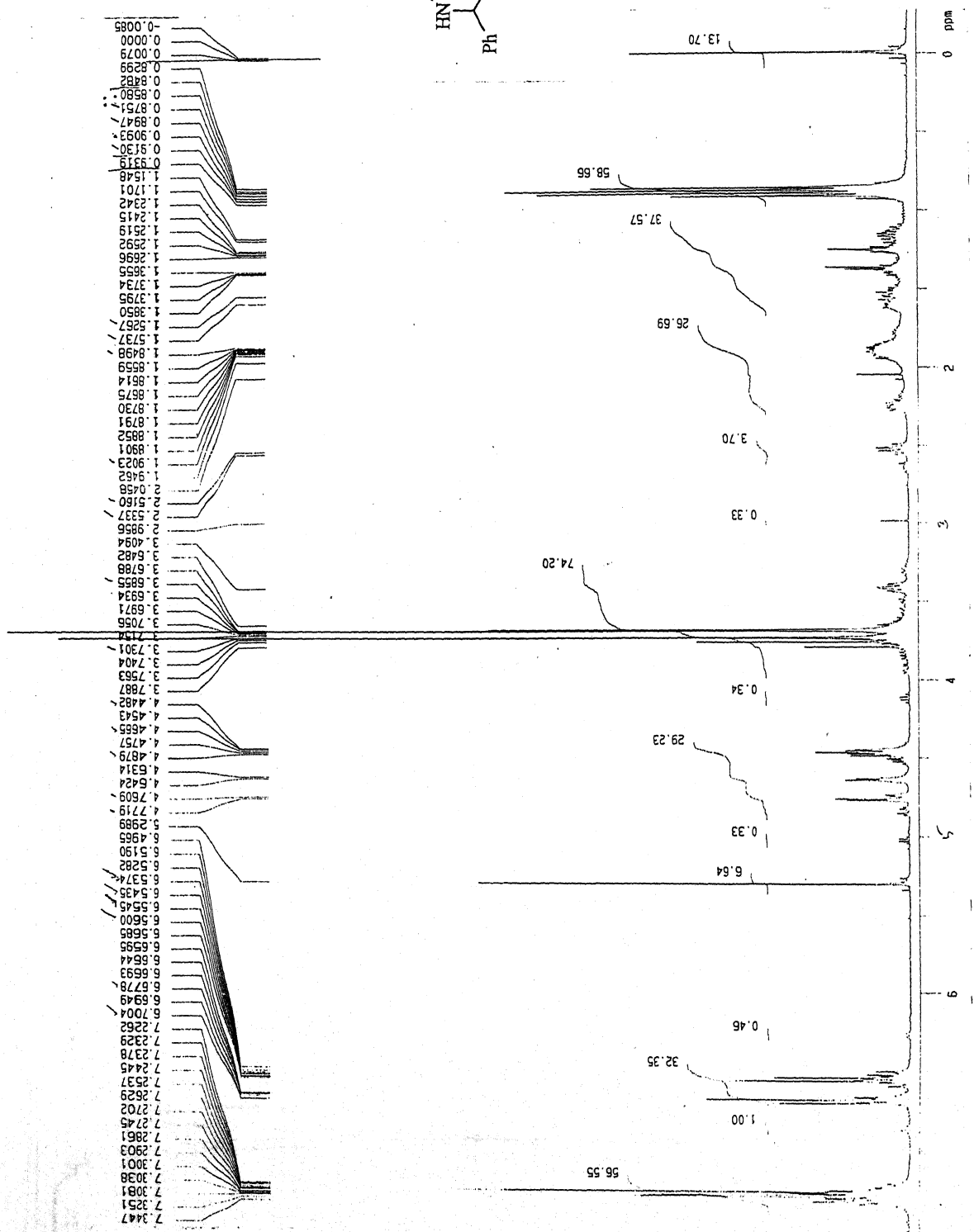
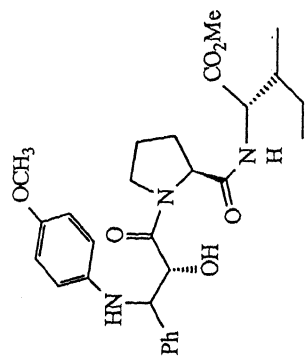
(m, 3H), 1.55 - 1.35 (m, 2H), 1.17 - 1.10 (m, 1H), 0.98 (d, $J = 6.6$ Hz, 3H), 0.89 (t, $J = 5.6$ Hz, 3H)

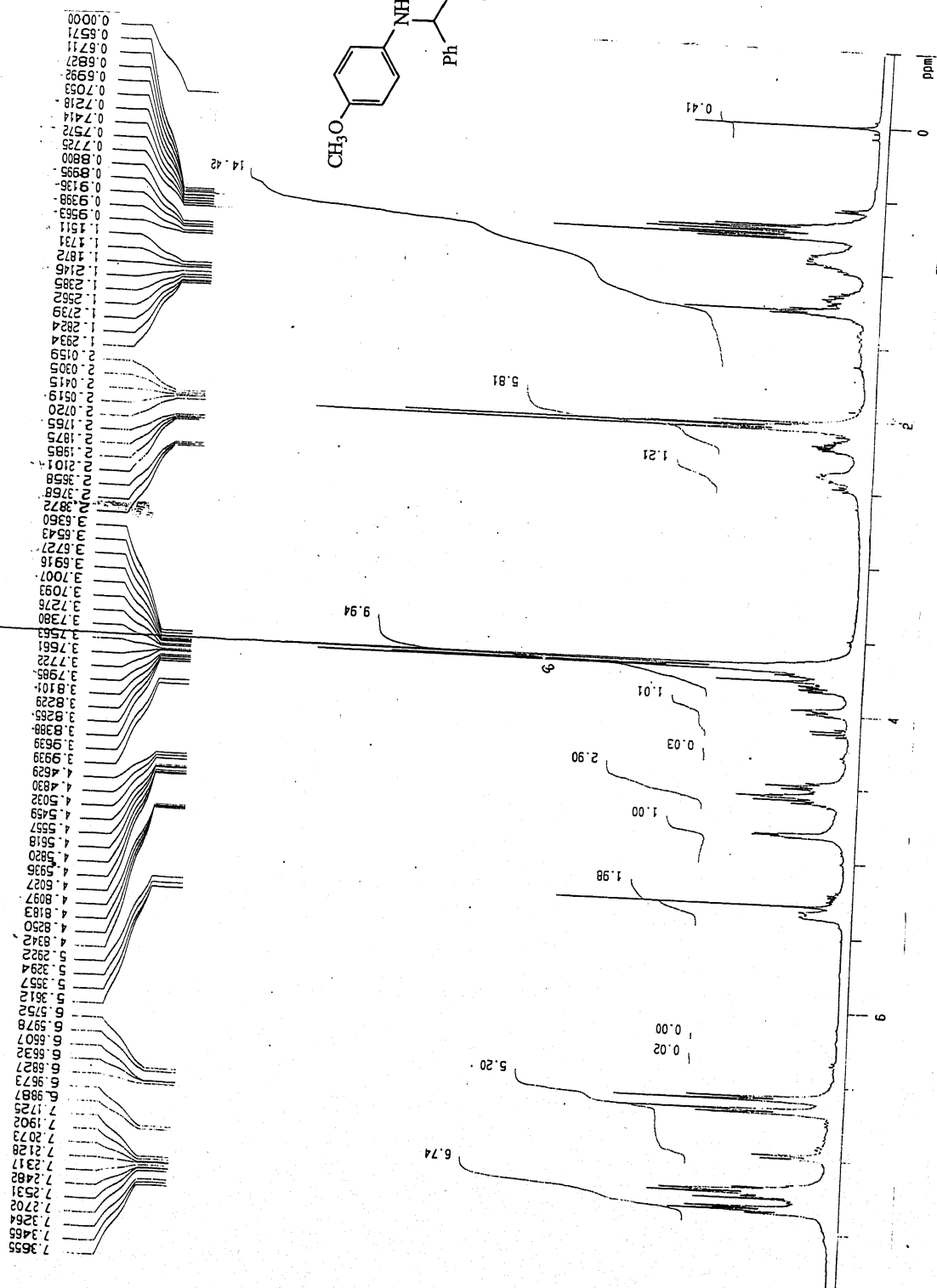
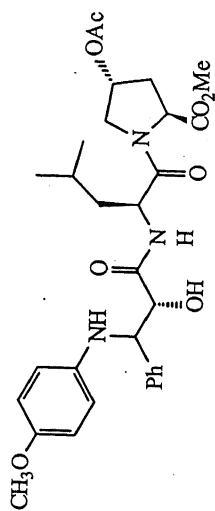
Synthesis of methyl-N,N-(phenyl(*m*-hydroxy-*p*-methoxybenzyl))- β -phenylisoserine-proline-leucinate (15**)**

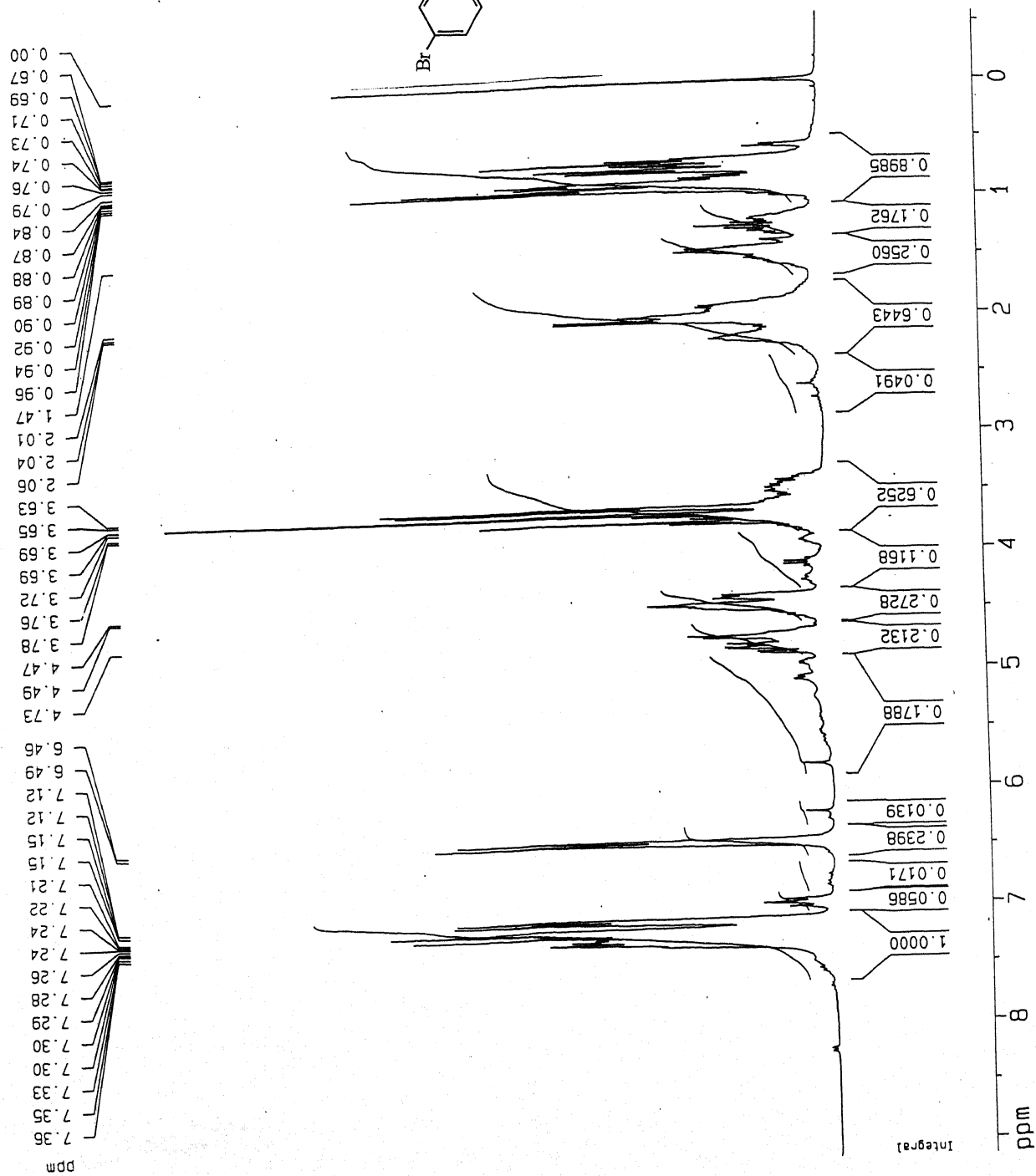
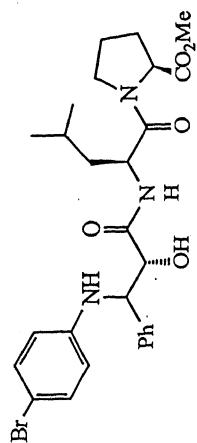
To a solution of methyl-N-cinnamoyl-proline-isoleucinate (558 mg, 1.5 mmol) in acetonitrile (7.5 mL), 2-methylpropanal (216 mg, 3 mmol) and PASCOS (~5mg) catalyst were added and the resulting solution was stirred under oxygen atmosphere for 12 h, after which a fresh lot of the catalyst and 2-methylpropanal (216 mg, 3 mmol) were added and the reaction was stirred to completion (TLC - $R_f = 0.5$ - EtOAc:Hexane - 1:1). After complete conversion of the olefin to the glycidic amide, the oxygen balloon was removed and a fresh lot of catalyst was added to the reaction mixture followed by the aromatic secondary amine N,N-phenyl-(*m*-hydroxy-*p*-methoxybenzyl)amine **18e**, (343 mg, 1.5 mmol). After stirring until complete consumption of the epoxide and formation of the product (TLC - $R_f = 0.2$ - EtOAc:Hexane - 7:3), washing of the unreacted material as described in the general procedure, with EtOAc:CCl₄:hexane gave the β -phenylisoserine derived peptide **15** in high purity (HPLC) and good yields (58 %), predominantly as the anti diastereomer.

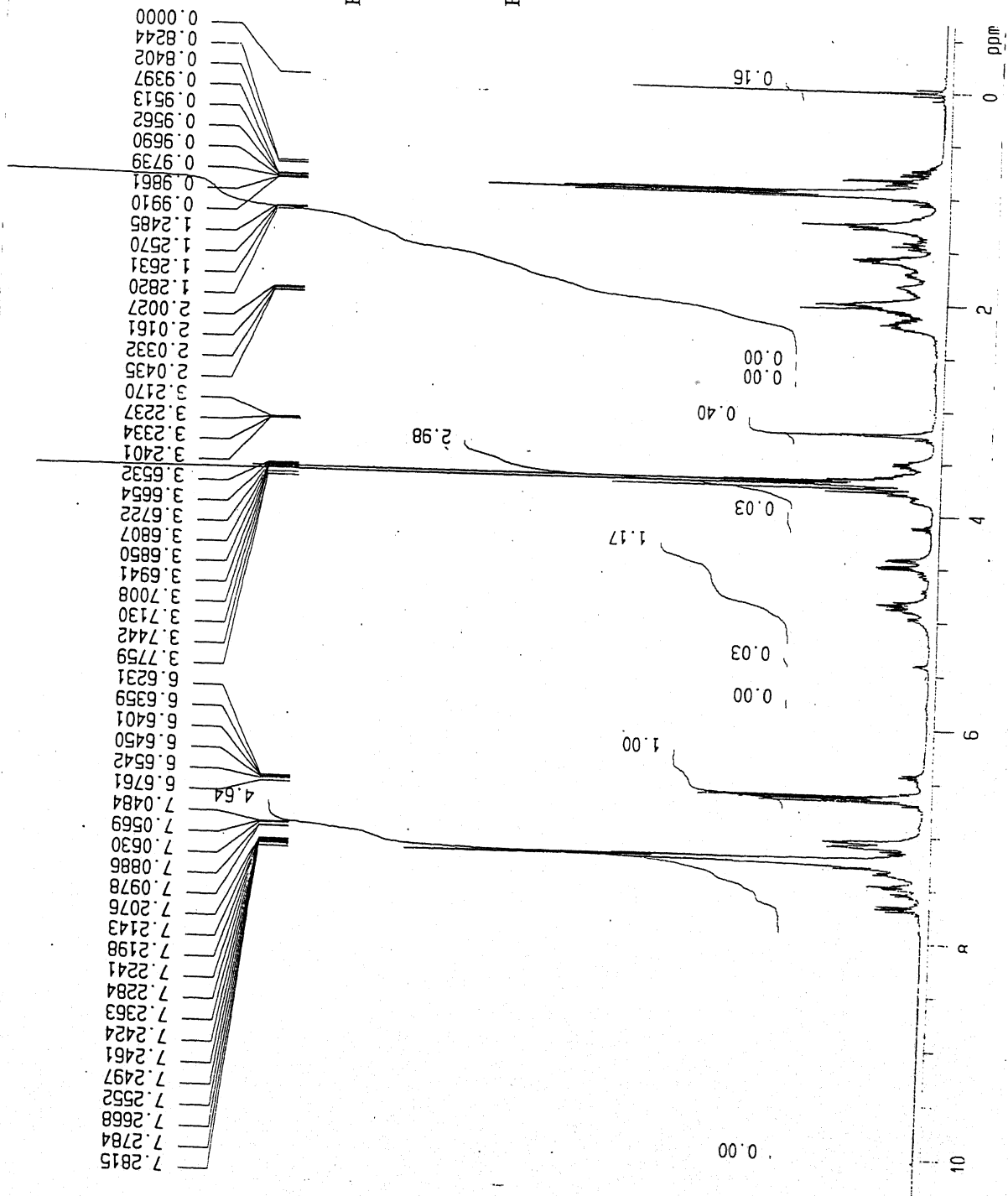
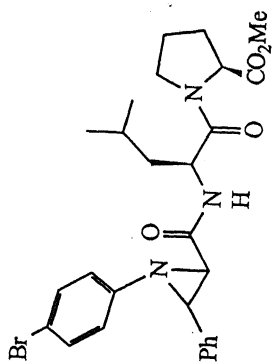
¹H NMR (CDCl₃) δ 7.30 (m, 1H), 7.22 (s, 5H), 6.81 (d, $J = 10.4$ Hz, 2H), 6.73 (d, $J = 10.4$ Hz, 2H), 5.88 (m, 2H), 5.43 (d, $J = 16$ Hz, 1H), 5.25 (d, $J = 8$ Hz, 1H), 5.16 (d, $J = 18$ Hz, 1H), 4.94 (d, $J = 3.4$ Hz, 1H), 4.86 (d, $J = 3.4$ Hz, 1H), 4.62 (m, 2H), 4.55-4.53 (m, 2H), 4.27 (d, $J = 17$ Hz, 1H), 3.82 (m, 1H), 3.69 (s, 3H), 3.60 (m, 1H), 3.52 (m, 1H), 2.19 (m, 1H), 2.05-2.02 (m, 3H), 1.65 (m, 1H), 1.41 (m, 1H), 0.98 (d, $J = 6.8$ Hz, 3H), 0.94 (d, $J = 5.5$ Hz, 3H)

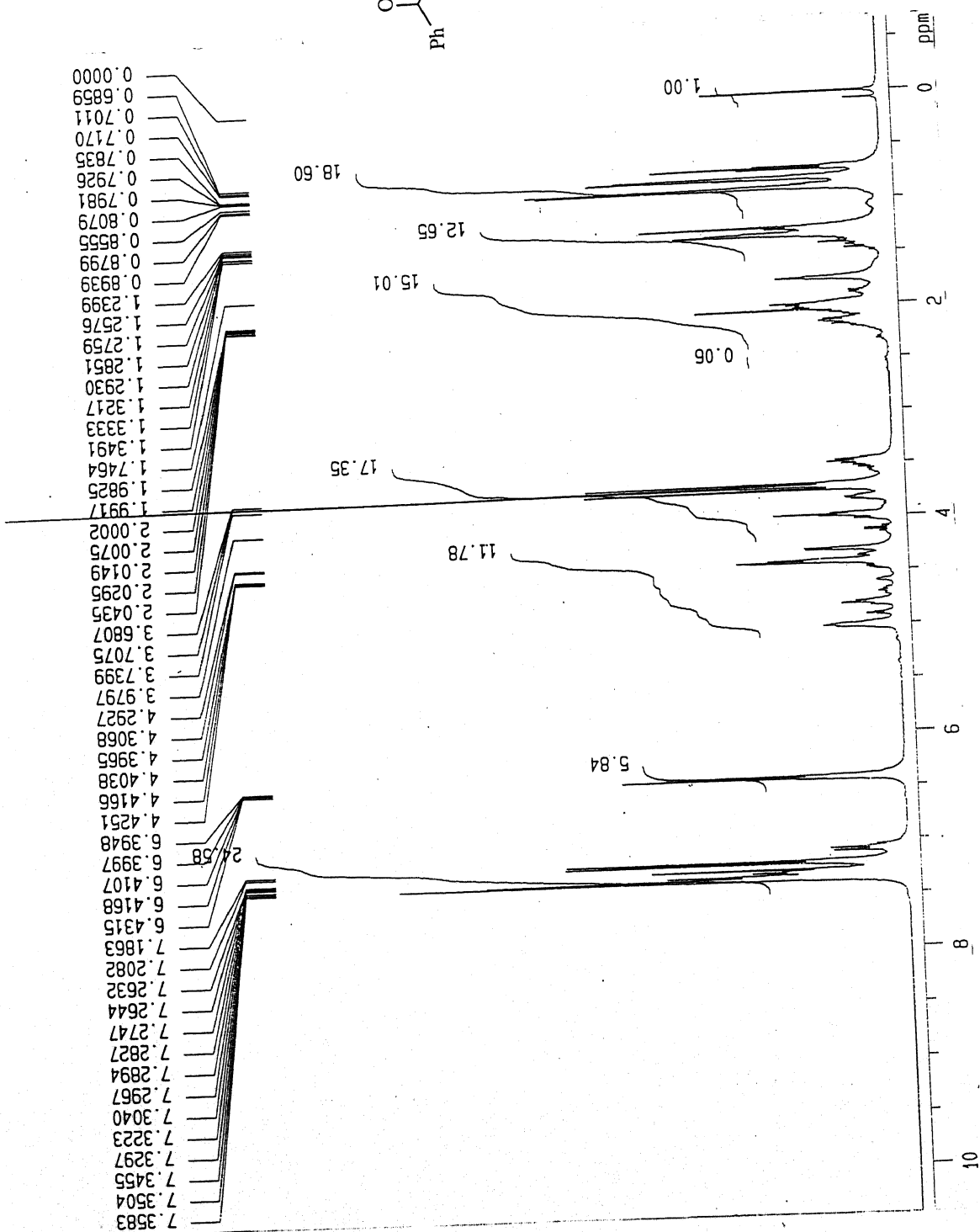


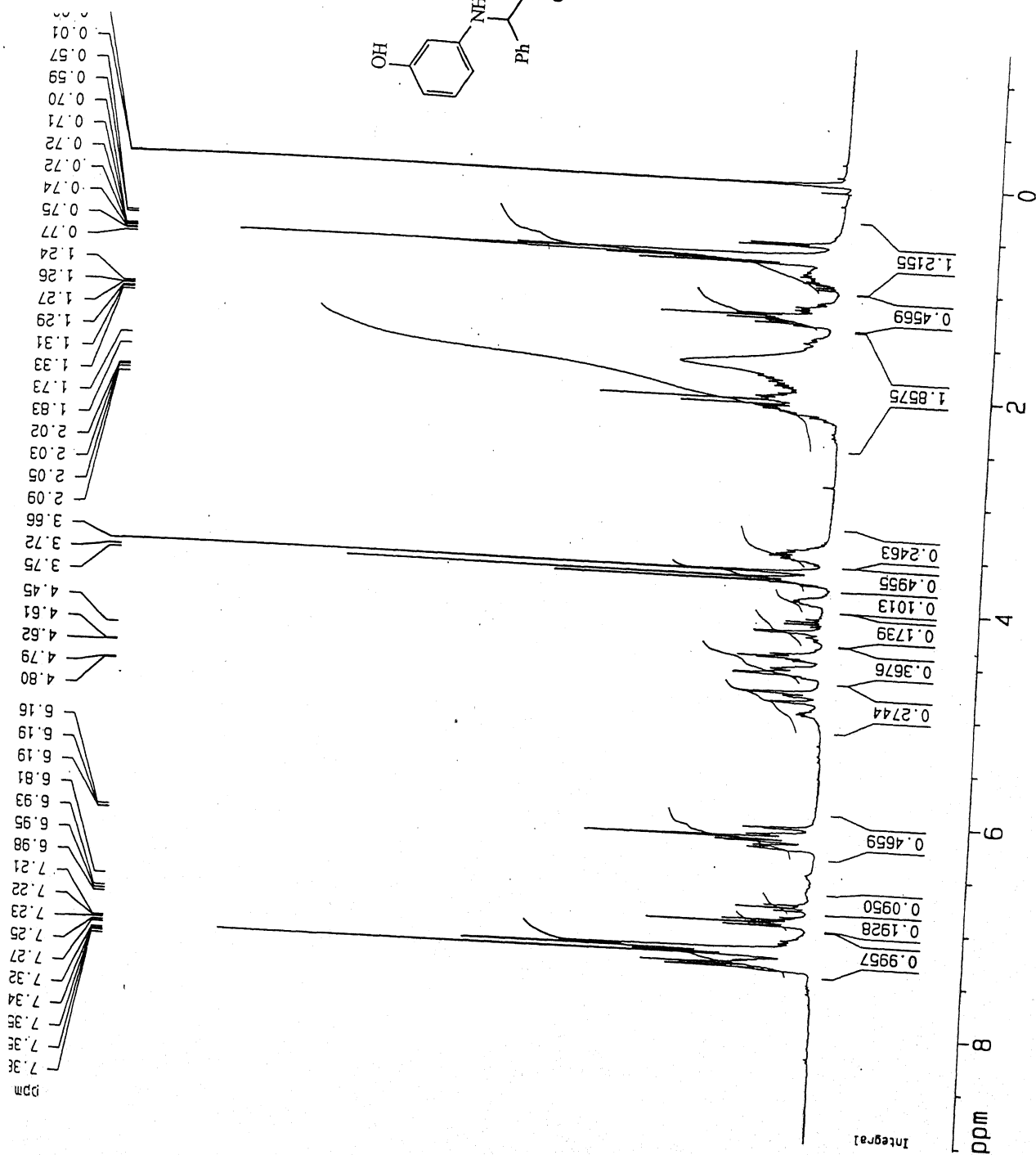
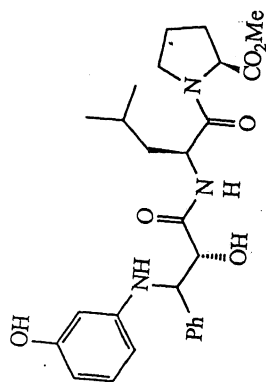


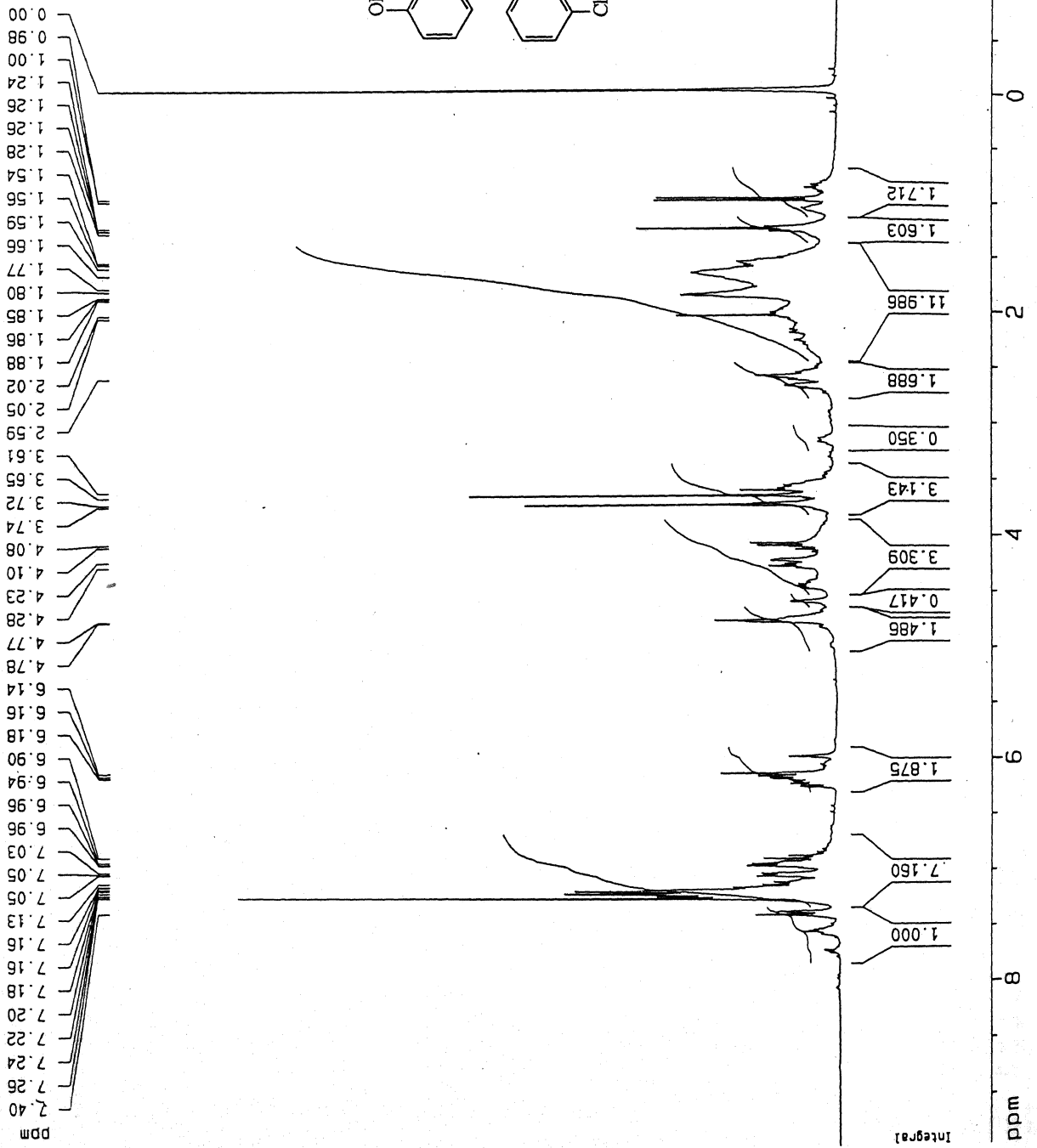
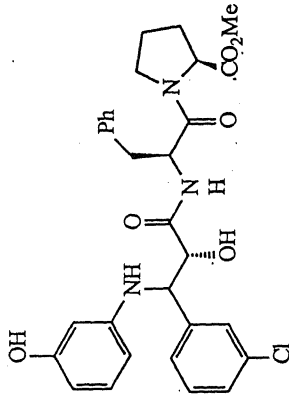


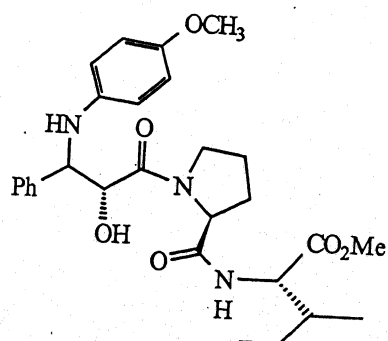
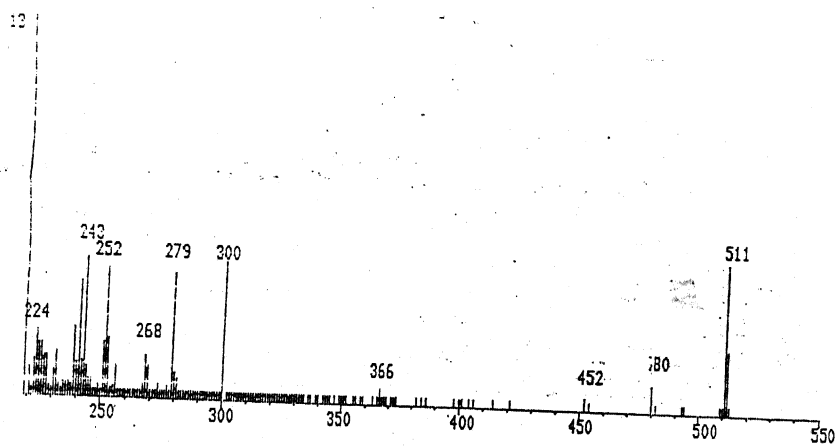
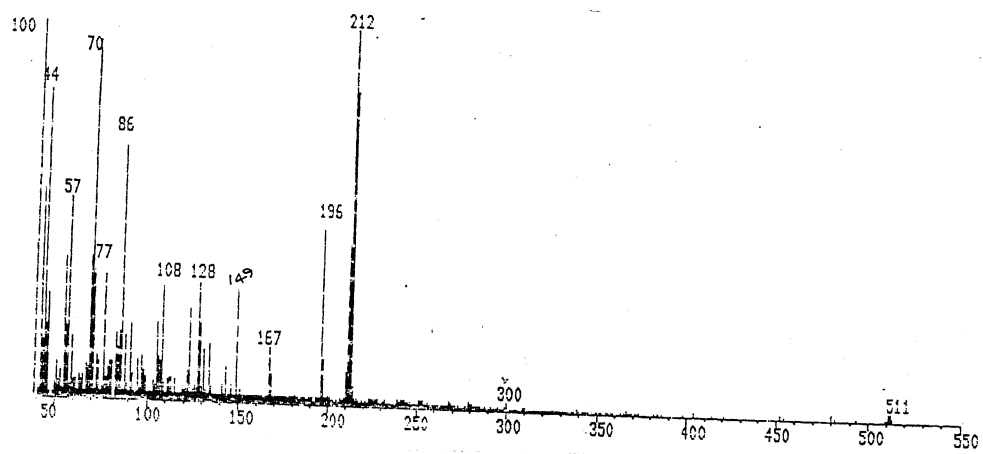


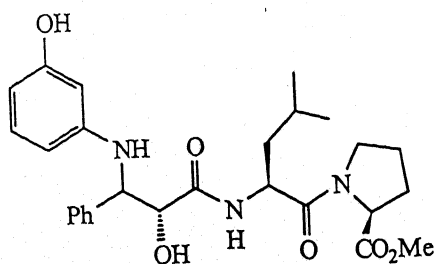
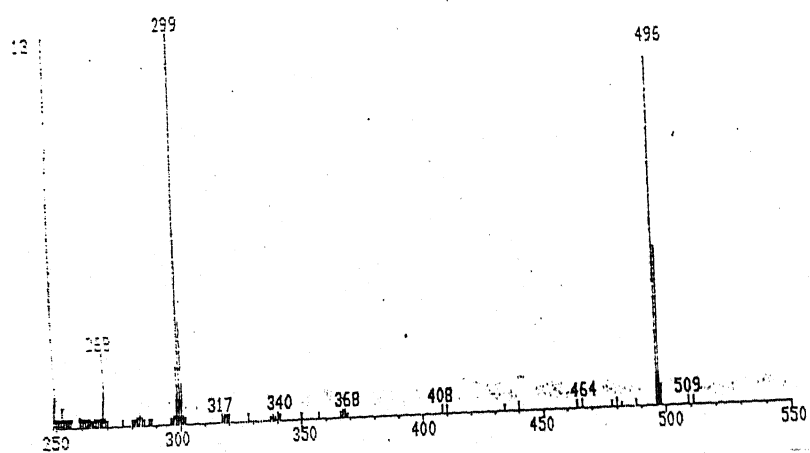
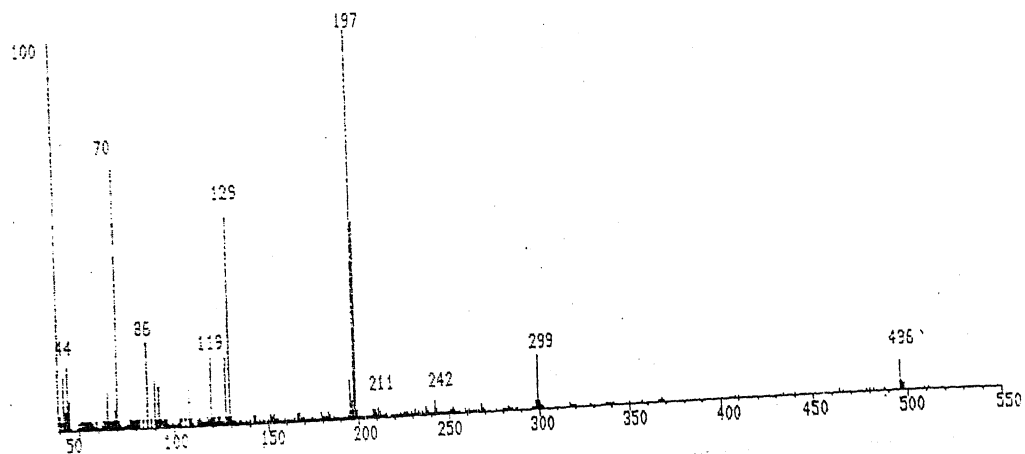


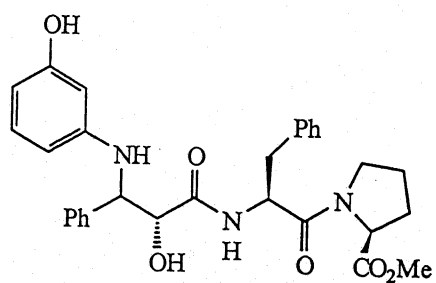
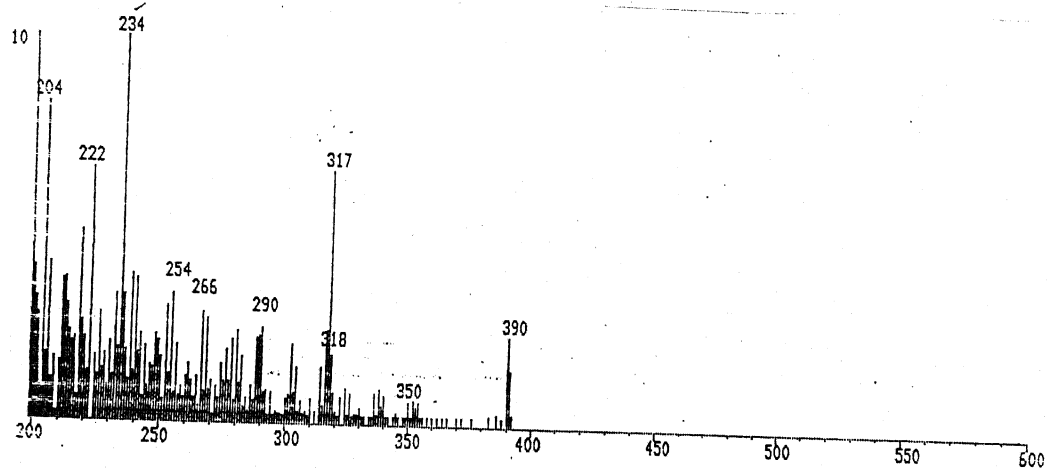
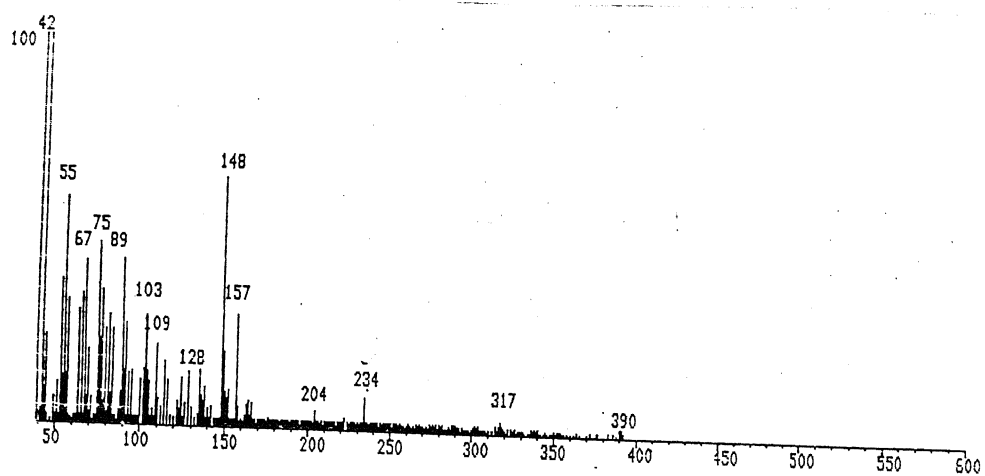












**CONFORMATIONALLY CONSTRAINED β -PHENYL
ISOSERINE DERIVED PEPTIDES VIA RING CLOSURE
METATHESIS**

Conformationally Constrained Peptides

The wide spread occurrence and extremely unfavorable prognosis of human immunodeficiency virus (HIV), the causative agent for the acquired immunodeficiency syndrome (AIDS) infection, has made the discovery of new and effective therapy very urgent.²⁶⁷ Of the currently licensed therapeutic agents which target the HIV reverse transcriptase, most have undesirable toxicities and select resistant mutant viral strains in a relatively short time.²⁶⁸ Another formidable problem is the seemingly limitless capacity of the AIDS retrovirus to evolve drug resistant mutations.²⁶⁹ Thus HIV PR, which is responsible for the maturation of HIV into infectious viral particles,²⁷⁰ has become an attractive therapeutic target. Starting from the strategy of mimicking the peptide chains around the "affected" substrate active site (substrate based design) and the introduction of isosteres for the scissile amide bonds, drug design has evolved numerous novel strategies for specifically attacking the enzyme active site in an effective manner.

Although replacing the scissile amide bond with a non-cleavable *cum* high-binding transition-state isostere usually gives potent protease inhibitors *in vitro*, the remaining hydrolysable amide bonds render the inhibitors unstable to peptidases. Attempts to replace them with retention of inhibitor potency have proved to be difficult,²⁷¹⁻²⁷⁴ due to the unpredictable co-operative influence of such variations on the conformations of both neighboring inhibitor groups and enzyme residues.

The search for the bioactive conformations of peptides at specific receptors, e.g. against HIV PR, thus faces a two-fold problem. The highly flexible nature of the linear natural peptides e.g. leucine, valine, methionine, etc. make them virtually structureless in solution and several different conformations (extended, single-bend, double-bend) have been observed in X-ray crystallography studies.²⁷⁵⁻²⁷⁷ In turn, the flexibility of these peptides renders them relatively non-selective in binding to the receptors. The problem is more complicated in designing certain drugs e.g. for binding to the opioid receptors, because of the discovery of these receptors with presumably subtle differences in binding requirements.²⁷⁸⁻²⁷⁹

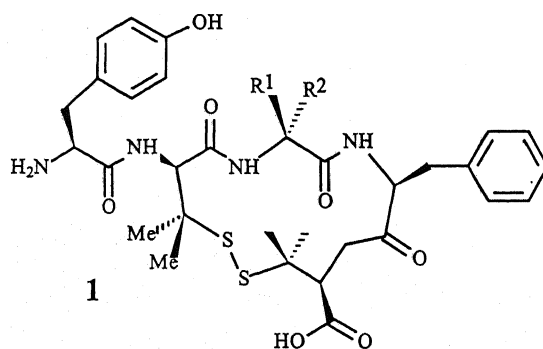
A central theme in many modern structure-function studies has been thus, the incorporation of conformational and topographical constraints into such linear peptides in order to force the structure into the binding conformation of the receptor, thus making it highly selective.²⁸⁰ The advances in computer aided molecular modeling have complemented this effort by predicting the spatial location of the required side chain

functional groups in such constrained conformers. The rest is left to organic chemists to come up with efficient synthetic methodologies to generate such molecules with functional diversities.

The success of such an approach is heightened by the fact that the enzyme-ligand complex, formed with the conformationally constrained ligand, is often more readily deducible by spectroscopic analysis (such as solution NMR and X-ray crystallography) which can facilitate the iterative synthesis of further peptide or peptidomimetic analogues targeted for improved binding and selectivity.

The other advantage of the constrained conformers over the acyclic peptides is that the amide bonds other than the isosteres in the constrained conformer are protected from enzymatic degradation, by making the amide bond less recognizable to other proteases that cleave them. This leads to greater bio- and oral-availability²⁸¹ and longer duration of action²⁸² of these drugs. These are in fact some of the most essential complimentary requirements in a drug lead, apart from its ability for active site binding.²⁸³ Constraining the conformations of acyclic molecules by their cyclisation has lead to success in design of many drug leads.

Hruby *et al.* reported²⁸⁴ the design and synthesis of the disulphide cyclised peptide analogue of a Leu⁵ (or Met⁵) enkephalin, [D-Pen², D-Pen⁵] enkephalin (H-Tyr-D-Pen-Gly-Phe-D-Pen-OH, DPDPE, Pen = penicillamine).



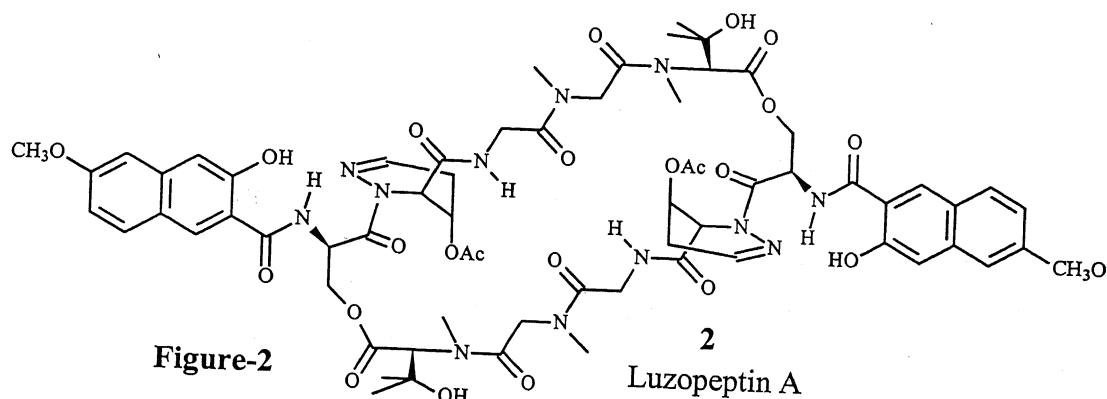
$R_1, R_2 = \text{H}$, H-Tyr-D-Pen-Gly-Phe-D-Pen-OH **DPDPE**

Fig. 1 Structure of [D-Pen², D-Pen⁵]enkephali

This cyclic constrained ligand was reported to be a potent opioid inhibitor, and selective to the δ -receptor among other receptor types, in a closely related family of such receptors with subtle secondary structural differences.

The Bristol-Banyu Research Institute in Tokyo reported²⁸⁵ the structure of a new natural product with a very high anti-tumor activity, named luzopeptin-A. Luzopeptin A, a

dimeric, cyclic depsipeptide is thought to interact with the DNA and thus play a role in its activity.



In an effort to synthesize potent thrombin inhibitors, based on the transition state analogue concept, the cyclic peptide CIA was designed²⁸⁶ from the acyclic inhibitor RWJ-50353 as a potent thrombin inhibitor. CIA contains a α -ketoamide isostere.

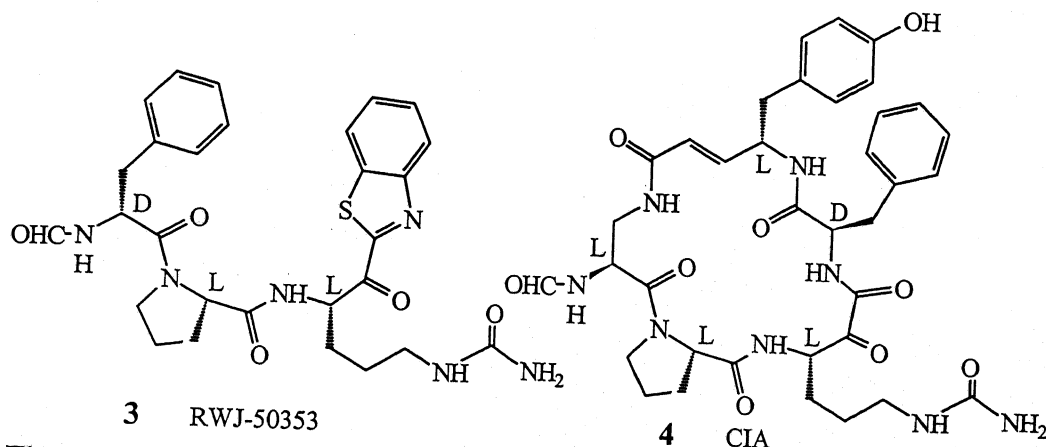


Figure-3 Acyclic and the Corresponding Cyclic Tripeptide Transition State Analogs Potent Thrombin Inhibitors

The development of cyclic aspartyl protease (e.g. renin, HIV-PR) inhibitors has been of recent interest.²⁸⁷⁻²⁹³ A novel class of potent macro cyclic renin inhibitors was synthesized through macrolactonisation.²⁸² These inhibitors incorporate the (2R, 3S)-3-amino-4-cyclohexyl-2-hydroxy-butanoic acid²⁹⁴ (nor-ACHPA) as the transition state isostere and have been seen to possess nanomolar inhibitory potencies against various aspartyl proteases.

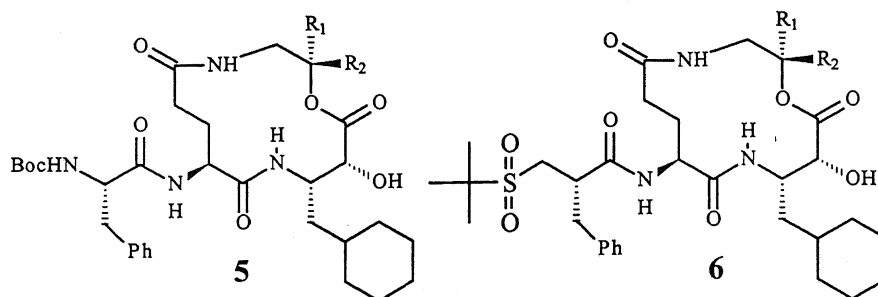
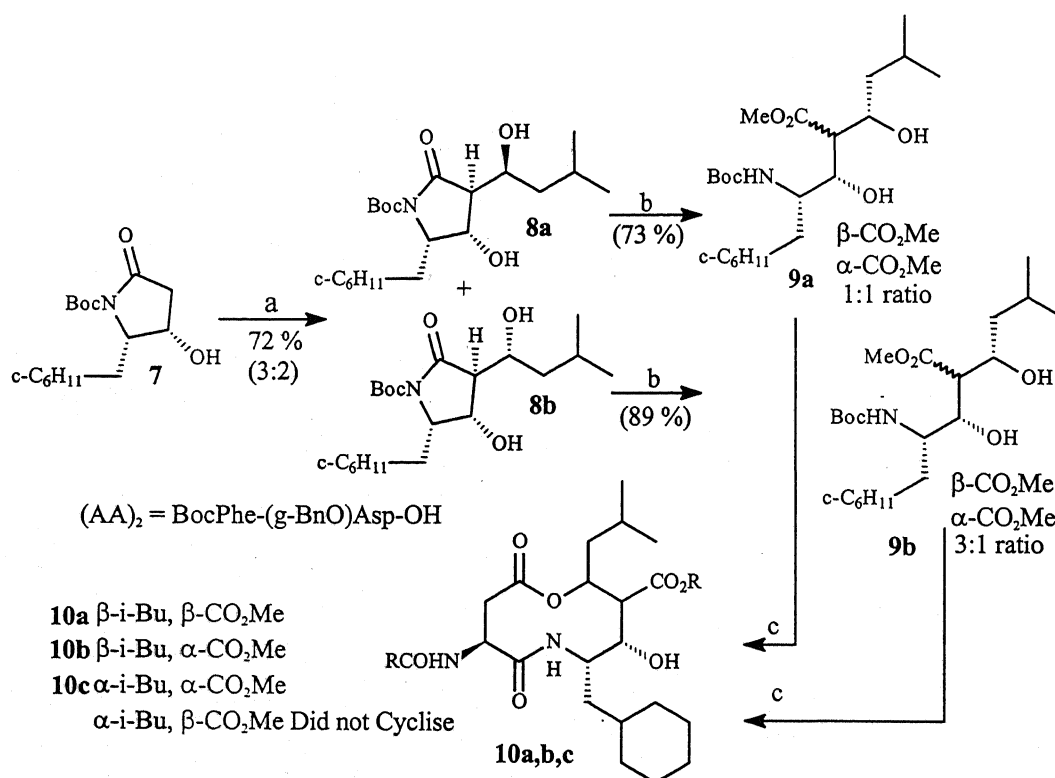


Figure-4 Macrocyclic Human Plasma Renin Inhibitors
Incorporating the *nor*-ACHPA isostere

From computer model studies of the active site of renin, another novel class of *nor*-ACHPA containing inhibitors was designed and synthesized.²⁹⁵



Scheme-1

Legend: (a) i, LDA/THF-HMPA, ii, i-BuCHO, iii, separate; (b) i, NaOMe/MeOH, ii, separate; (c) i, TFA/CH₂Cl₂, ii, activated (AA)₂, iii, H₂/Pd-C, iv, DCC/DMAAP

Cyclisation was effected by macrolactonisation. The highly stereochemically complex molecule was synthesized (Scheme-1).

In an effort to develop human renin inhibitors with "improved bioavailability", macrocyclic renin inhibitors^{288,290,296-299} **11** and **12**, containing an analogue of ACHPA, which incorporates a 2(S)-4-butenyl chain, were designed and its synthesis reported.³⁰⁰

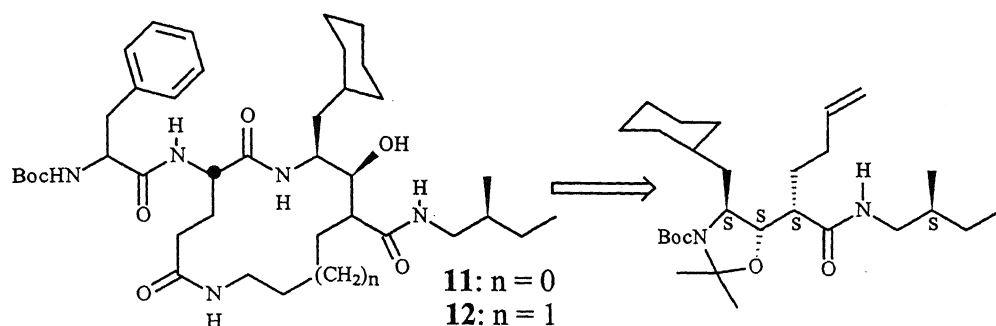


Figure-5 Macrocyclic Human Renin Inhibitor Incorporating ACHPA analog
Containing a 2(S)-4-butenyl chain

Another novel series of tripeptides with 14- to 16-membered rings containing urea linkage with high non-degradability (stable to the chymotrypsin degradation) were reported.²⁹⁰ Following the report of a novel class of renin inhibitors, characterized by the presence of a glycol³⁰¹ function replacing the scissile amide bond of angiotensinogen (a prototypical substrate, cleaved by renin), was synthesized with high binding potency ($IC_{50} = 1.5$ nM) but very facile degradation by chymotrypsin (the serine protease). The reported cyclic peptides were found to be highly potent and stable to chymotrypsin degradation.

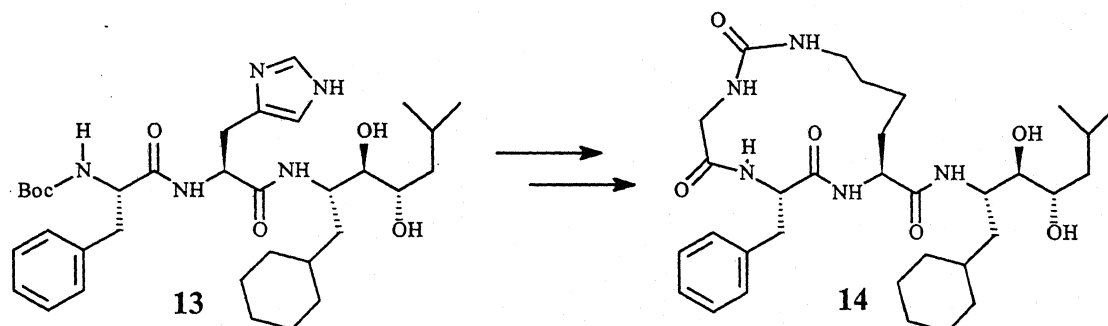
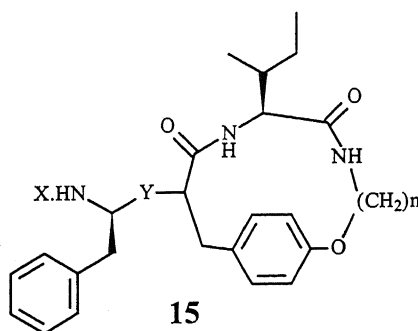


Figure-6 Acyclic Antihypertensive Renin Inhibitor
Incorporating a Glycol Isostere

Figure-6 Novel Small Cyclic Peptide Stable to
Chymotrypsin Degradation

Fairlie *et al.* have evolved new strategies for developing peptidomimetics involving the replacement of the flexible segments of peptide substrates with conformationally constrained hydrolytically stable, macrocyclic, structural mimics.²⁸¹ Modifications in the chain length and isostere of the competitive inhibitors Ac-Ser-Leu-Asn-Phe-{CHOHCH₂}-Pro-Ile-Val-NH₂ (JG 365), a potent heptapeptide inhibitor of HIV PR,²⁶¹ led to the design of hexapeptide Ac-Leu-Val-Phe-{CHOHCH₂}-Phe-Ile-Val-NH₂. From the receptor bound X-ray crystal structure of the acyclic peptide inhibitor, a cyclic inhibitor was designed containing a C-terminal macro cycle as a structural mimic that locks the otherwise flexible C-terminal peptide into a protein binding conformation²⁸¹ and

is stable to hydrolysis and proteolysis. The novel macro cycle superimposed well on the linear peptide inhibitor for which it was designed as a structural mimic. Structural mimicry led to functional mimicry as shown by comparable inhibition of the protease by cyclic and acyclic molecules.



| 15 | Compound* | X | Y | n | K _i nM |
|----|--------------------------------------|----------------------------------|---------------------------|---|-------------------|
| a | Ac-SLNFφPIV-NH ₂ (JG-365) | - | - | - | R 13 |
| b | Ac-LNFφPIV-NH ₂ | - | - | - | S 1 |
| c | Ac-LVFφFIV-NH ₂ | - | - | - | R 18 |
| d | | Ac-Leu-Val | -CH(OH)CH ₂ NH | 3 | S 1 |
| e | | Ac-Leu-Val | -CH(OH)CH ₂ NH | 4 | R 1 |
| f | | Boc | -CH(OH)CH ₂ NH | 3 | R-S 1.5 |
| g | | Tetrahydrofuryl-3(S)-oxycarbonyl | -CH(OH)CH ₂ NH | 3 | R 0.6 |
| h | | QC-val | -CH(OH)CH ₂ NH | 3 | R 1.5 |

Table-1 Inhibition of HIV-1 Protease by Cyclic Peptidomimetics

QC = quinoline(2)-carbonyl; Ac = acetyl;

* = refer mnemonic aids

Molecular modeling and X-ray crystal studies showed that the cyclic inhibitors bind uniquely to the HIV1-PR. The sterically encumbered hydroxyethylamine isostere bound via both its hydroxyl and protonated nitrogen to the anionic Asp₂₅ catalytic residues. Of all the N-terminal substituents, the longer quinoline containing peptide **15h**, which can make more interactions with the HIV PR, increased potency significantly. In each of the cases, the acyclic peptidic inhibitors (**15a-c**), of HIV PR were completely degraded under the same conditions and showed no anti-viral activity at 10 μM concentrations.

In their earlier report, Fairlie *et al* had reported the regioselective structural mimicry of the "N-terminal" tripeptide fragment -Leu-Asn-Phe- (of the potent linear hexapeptide **15a**, Table-1), containing the hydroxyethylene isostere) by using spacers to fix the conformations of the native amino acid sequence.³⁰² Hydrogen bonds between HIV PR

and **15a** were comparable to those in the enzyme complex of (JG 365), including the structural water mimicry of the main chain amide carbonyls.³⁰³

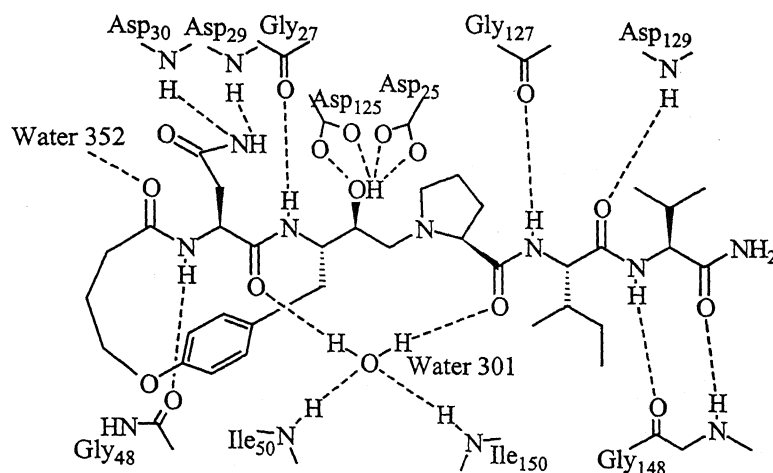
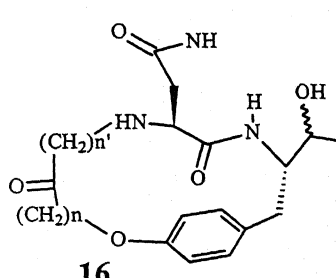


Figure-7 Schematic representation of X-ray crystal structure of N-terminal-constrained inhibitor, bound to HIV PR, showing hydrogen bonds to the enzyme

But the main advantage of cyclic over the acyclic conformers was the observation that the cyclic conformers were highly hydrolytically and proteolytically stable than their linear counter parts, hence imparting the much needed bio- and oral-availability for use as better drug candidates.

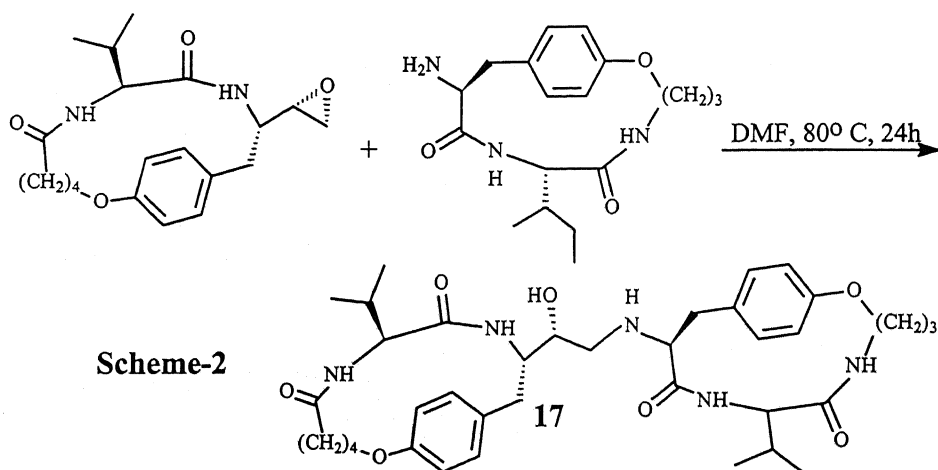
| | S.No. | n' | n | R/S | Y | K _i (nM) ^a |
|-------------------------------------------------------------------------------------|-------------|-----------|---|-----|---------------------|----------------------------------|
|  | 16 a | 0 | 3 | S | PIV-NH ₂ | 12 |
| | b | 0 | 4 | S | PIV-NH ₂ | 8 |
| | c | 0 | 3 | R | Pip-N-tBt | 5 |
| | d | 0 | 4 | R | Pip-N-tBt | 17 |
| | e | 0 | 4 | R | Cycle (5) | 3 |
| | f | -Gly- | 1 | S | PIV-NH ₂ | 11 |
| | g | -Tyr- | 1 | S | PIV-NH ₂ | 11 |
| | h | -(D)Gln-1 | | R/S | PIV-NH ₂ | 4 |
| Ac-LeuAsnPhe(HEA)ProlleVal-NH ₂ S 3 | | | | | | |

a) pH 6.5, I = 0.1 M, 37°C, 50 μM substrate [Abz-NF*-6], synthetic HIV-1 PR.
Bergman, D.A. et al, *Letters in Peptide Science* 1995, 2, 99

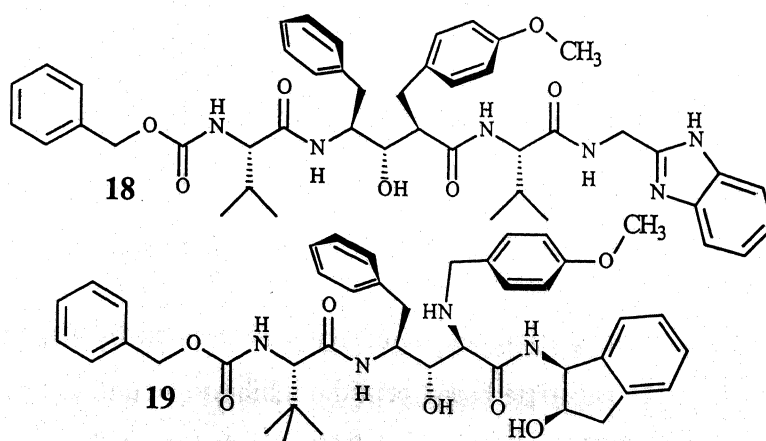
Table-2 Inhibition of HIV PR by Cyclic Peptidomimetics

In another report,¹⁶⁸ a tandem approach of design was applied by Fairlie. The X-ray crystal structures of twelve substrate based peptidic inhibitors bound in the active site of the aspartyl protease (HIV PR) were compared. The remarkable similarity in the inhibitor binding modes of these structurally and conformationally diverse inhibitors prompted the design of a bicyclic peptidomimetic inhibitor **17** with macrocyclic components in

constrained conformations that are pre organized for receptor binding. A series of appended macrocycles were synthesized and tested as inhibitors of HIV1-PR.³⁰³ Structure activity relationships were explored by systematic replacement of the individual amino acids and the results yielded the bicyclic appendage ligand **17**, as the most potent inhibitor (nanomolar, against HIV PR)



Peter Ettmayer *et al* have come up with a novel class of water-soluble compounds, namely the cyclophanes. Cyclophanes constitute a novel building block for potent HIV 1 PR inhibitors.³⁰⁴ These are macrocycles with one or more aromatic rings incorporated; ansa compounds. Several derivatives of K_i values lower than 10 nM and antiviral activities in the range of 15 - 50 nM have been obtained. Some of the most potent compounds are presented below.



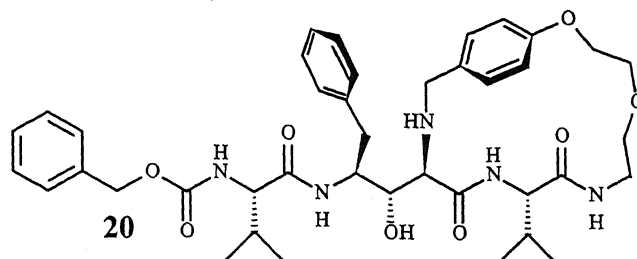
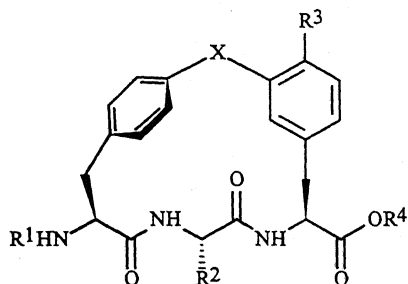


Figure-8 Structure of cyclophane **20** and its lead:
Potent Inhibitors of HIV PR

Rich *et al* reported another such cyclophane. A novel mechanism based cyclic biphenyl ether peptide β -strand mimetic and HIV PR inhibitor was designed and synthesized.³⁰⁵ Compounds **21** and **26** containing the hydroxyethylamine isostere emerged from these studies as highly potent ($K_i = 15, 900$ nM respectively) inhibitors of HIV PR.

Novel Cyclic Biphenyl Ether Peptide β -Strand Mimetics and HIV-PR Inhibitors



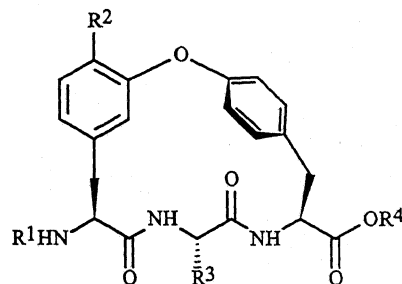
21 $R^1 = \text{Ac}$; $R^2 = 4\text{-OH-Bn (Tyr)}$; $R^3 = \text{OH}$; $R^4 = \text{H}$; $X = \text{O}$, **K-13**;

22 $R^1 = \text{Ac}$; $R^2 = 4\text{-OH-Bn (Tyr)}$; $R^3 = \text{OH}$; $R^4 = \text{H}$; $X = \text{S}$

23 $R^1 = \text{Boc}$; $R^2 = s\text{-butyl (Ile)}$; $R^3 = \text{H}$; $R^4 = \text{CH}_3$; $X = \text{O}$

24 $R^1 = \text{H.HCl}$; $R^2 = \text{Bn (Phe)}$; $R^3 = \text{H}$; $R^4 = \text{CH}_3$; $X = \text{O}$

25 $R^1 = \text{H.HCl}$; $R^2 = s\text{-butyl (Ile)}$; $R^3 = \text{H}$; $R^4 = \text{CH}_3$; $X = \text{O}$



26 $R^1 = \text{H}$; $R^2 = \text{OH}$; $R^3 = \text{CH}_2\text{CONH}_2$; $R^4 = \text{H}$;

OF4949-IV

27 $R^1 = \text{CBz}$; $R^2 = \text{H}$; $R^3 = s\text{-butyl (Ile)}$; $R^4 = \text{CH}_3$

28 $R^1 = \text{H.HCl}$; $R^2 = \text{H}$; $R^3 = s\text{-butyl (Ile)}$; $R^4 = \text{CH}_3$

Table-3

Novel computational approaches to the problem of designing non-peptide HIV PR inhibitors have lead to the discovery of haloperidol³⁰⁶ and benzophenones.^{23,307} Such an approach has been most efficiently used for the synthesis of a novel class of cyclic urea derivatives.³⁰⁸ It represents one of the best examples for the efficient design of conformationally constrained non-peptide HIV PR inhibitors. The computational analyses employed the following key ligand-substrate interactions in the design.

1) Displacement of the structural water molecule in the HIV1-PR with suitable groups with in the ligand for entropically favored, greater binding. The urea carbonyl replaced^{272,309} the structural water in the HIV PR; 2) Presence of C_2 -axis of symmetry in the ligand, since HIV PR is unique in functioning as a homodimer with a C_2 -axis of

symmetry; 3) Presence of the hydroxyl group for interaction with the Asp₂₅ and Asp_{25'} of the HIV PR; 4) Presence of P_n and P_{n'} hydrophobic groups for complementary binding with the HIV protease active site; and 5) Suitably cyclizing the molecule in order to provide a high degree of structural pre-organization.

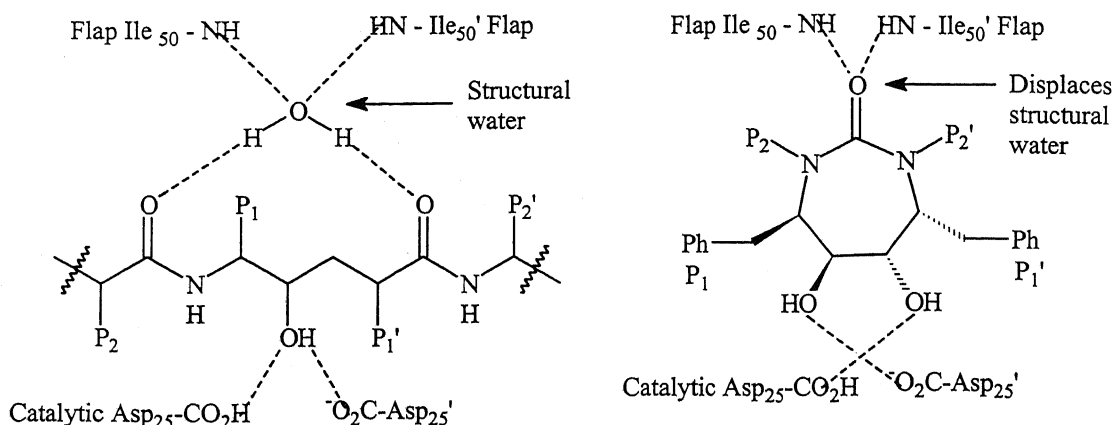
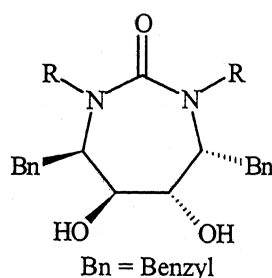


Figure-9 Peptidomimetic inhibitor binding at HIV-PR active site via Structural Water; and the Structurally Preorganised Cyclic Urea Inhibitor at the active site showing the urea oxygen displacing structural water

Thus starting from the first report of cyclic urea derivatives as potent inhibitors of HIV PR by Lam *et al.*,³⁰⁹ huge libraries of cyclic urea derivatives of varying ring size;

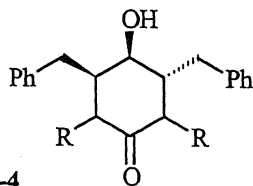


29 DMP 323 R = 3-(hydroxymethyl)benzyl.
30 DMP 450 R = 3-aminobenzyl

Figure-10

containing diverse hydrophobic complementary groups; and amide N-substituents have been synthesized. The most potent of them were **DMP 323**, **DMP 450**.

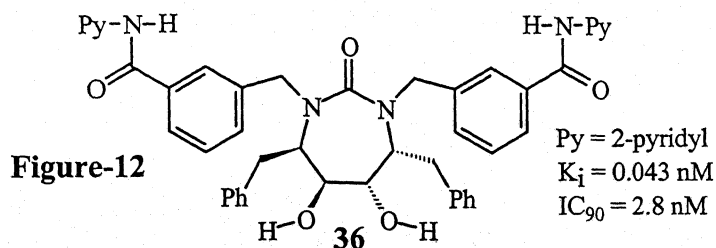
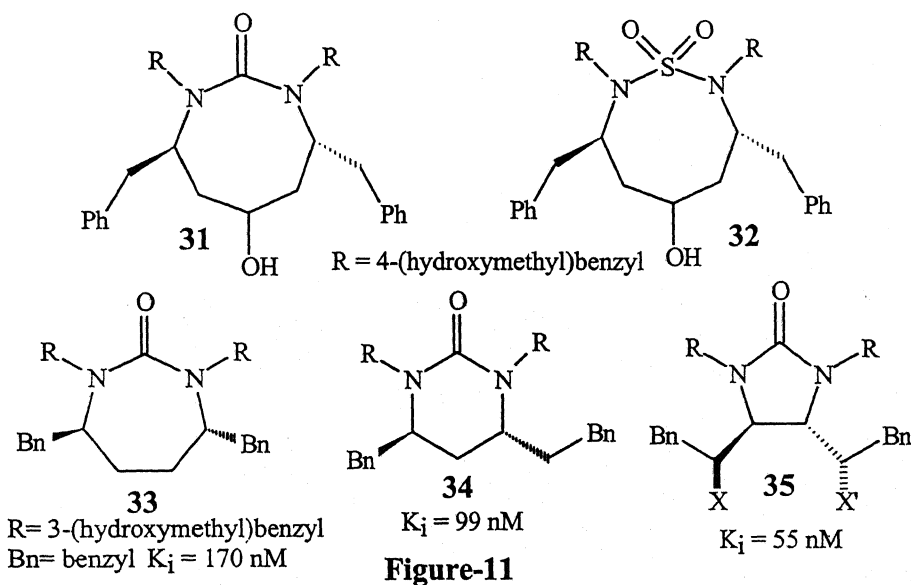
The 6-membered cyclic urea with decent inhibitory activity against HIV PR was synthesized based on the acyclic C₂ symmetric HIV PR inhibitor A-74704.³¹⁰

**Table-4**

| Compound | R | IC ₅₀ (μM) |
|----------|--------------------------------------|-----------------------|
| 31 | H | >100 ^a |
| 31a | PhCH ₂ - | 89 |
| 31b | CH ₂ =CHCH ₂ - | 53 |
| 31c | cyclopropyl methyl | 9 |

a) Inhibition of HIV PR was measured using flurogenic substrate under assay conditions as in Kageyama, S. *et al* *Antimicrob. AgentsChemother.* 1993, 37, 272

A study on the effect of ring size and other cyclic scaffolds on the inhibitory activity of the cyclic series of HIV PR inhibitors led to the synthesis of 8-membered cyclic urea & cyclic sulphanamides³¹⁰ and 5-, 6- and 7-membered imidazolidinones.³¹¹



Based on the structural studies on DMP 323, a 7-membered cyclic oxanamide³¹² and a cyclic urea³¹³ have been synthesized containing large, hydrophobic, Pn, Pn' side chains, as potent HIV PR inhibitor.

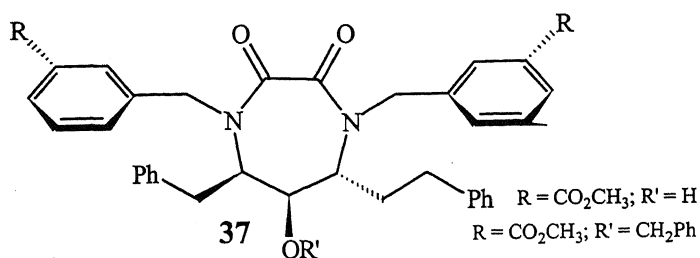


Figure-13 7-Membered cyclic oxamides: Novel HIV-1 PR inhibi

Subsequently a novel picomolar azacyclic urea inhibitor has been reported.²⁶⁷

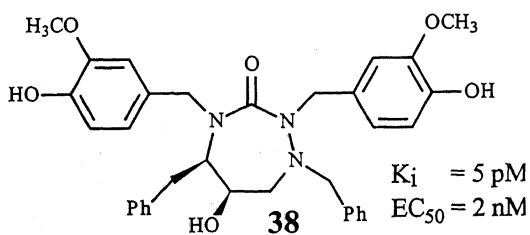


Figure-14 Azacyclic urea: HIV PR Inhibi

Oral bioavailabilities of these inhibitors increased on the introduction of non-symmetrical amide substituents.³¹⁴ δ -lactam derivatives have also been reported as potent HIV PR inhibitors.³¹⁵

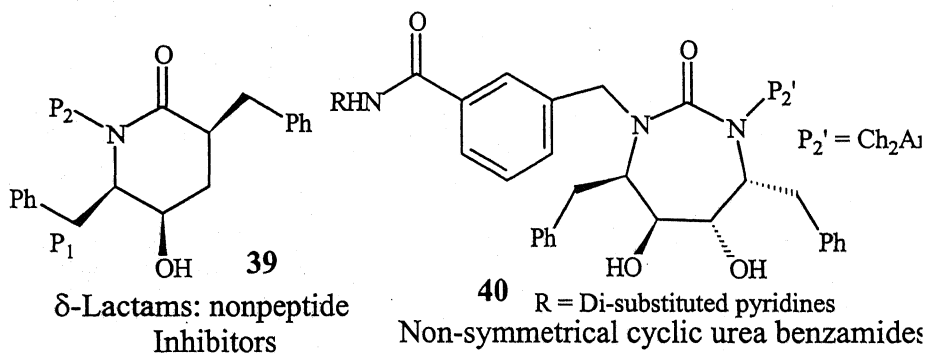
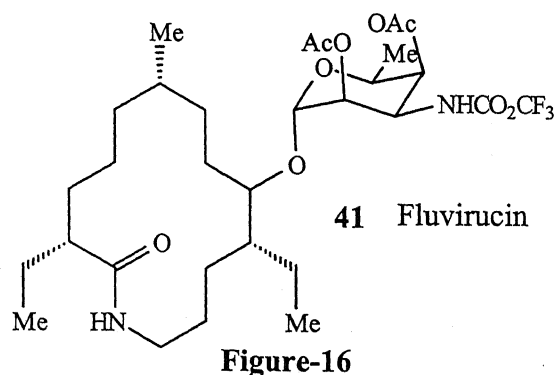


Figure-15

Ring Closure Metathesis

Transition metal catalyzed C-C bond formations belong to the most important class of reactions in organic synthesis. Almost every kind of transformations and covalent bond formations have been achieved by transition metal catalyzed reactions. The total synthesis of fluvirucin **41** by Hoveyda *et al*³¹⁶⁻³¹⁷ is a classic example, which includes no less than nine transition-metal catalyzed reactions.



One particularly interesting of such metal-catalysed reactions is olefin metathesis, a metal catalysed exchange of alkylidene moieties between alkenes (For a brief history and mechanism of olefin metathesis, see references 318-335).

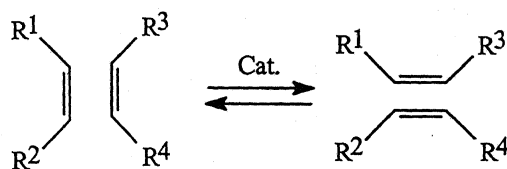


Figure-17 General Principle of olefin metathesis

Olefin metathesis can induce both cleavage and formation of C-C double bonds. Special functional groups are not necessary. Although this reaction did not get familiar among organic chemists for many years, the recent abrupt end to this “Sleeping-Beauty” slumber³³⁶ has been due to the development of novel catalysts that can effect the conversion of highly functionalized and sterically demanding olefins under mild reaction conditions and in high yields. From the synthesis of fine-tuned polymers to the synthesis of various small molecules, olefin metathesis is very common in most organic syntheses today. A cursory glance at the current synthetic literature attests to the explosion in the application of metathesis.³³⁷⁻³³⁸ The highly developed ring closure metathesis has been proven to be the key step in the synthesis of a growing number of natural products. The number of applications of this reaction has dramatically increased in the past few years. Particularly, since this transformation utilizes no additional reagents beyond a catalytic amount of

metal carbon and the only other product from the reaction is, in most cases, a volatile olefin such as ethylene.

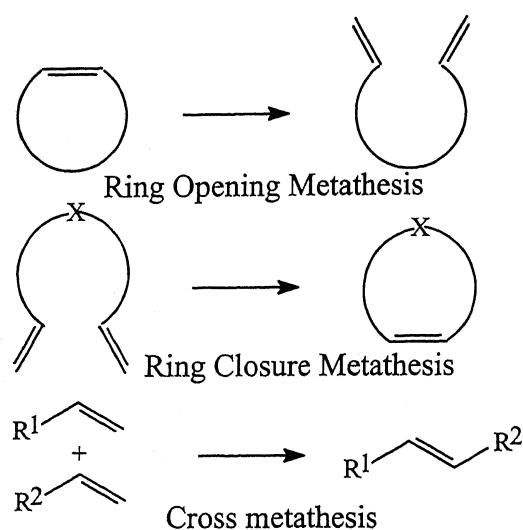
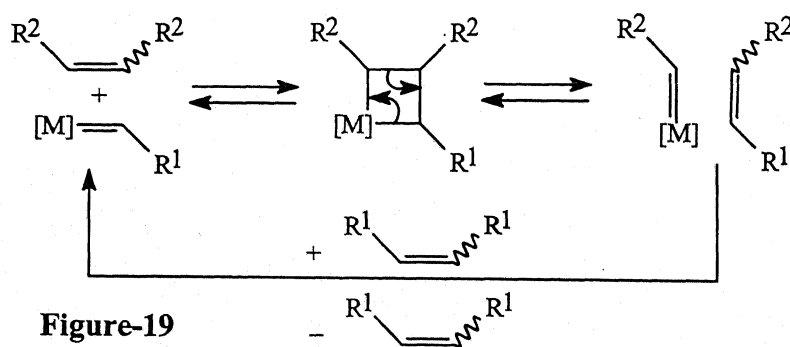


Figure-18 Metal Catalysed Metathesis Reaction

Among various metal catalysed metathetic reactions, of present interest to us is the transition metal catalysed ring closure olefin metathesis (RCM). The mechanism of both cyclic and acyclic olefin metathesis proceeds through a series of metallocyclobutanes and carbene complexes.³³⁹⁻³⁴¹



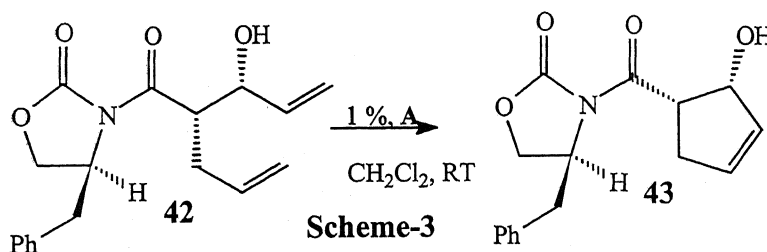
Mechanism of Olefin metathesis according to the Chauvin model

Recently, ring closure metathesis (RCM) has received a great deal of attention for the synthesis of medium or large sized rings from acyclic diene precursors. This intensive study is primarily due to the development of well-defined metathesis catalysts that are tolerant to many functional groups and also reactive towards a diverse range of substrates. Whether a polymer or cyclic compound is formed from any given diene is most often determined by thermodynamic rather than kinetic factors.³⁴²

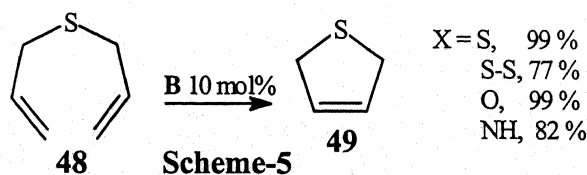
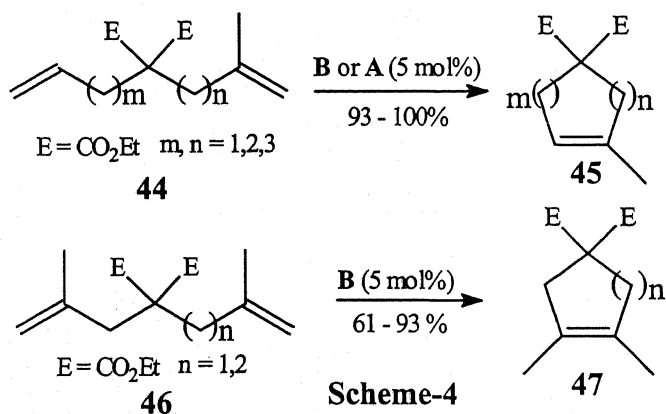
(ADMET) and ring-closing reactions depends on ring size, dilution, substrate and catalyst.

RCM Cyclizations

In general, four-membered rings cannot be obtained, while five- to seven-membered rings form readily due to their relatively low ring strain. Due to its higher tolerance towards substituents / functional groups and greater stability, Grubb's ruthenium complex **A** is a valuable resource. The cyclopenteneol derivative **43**, a precursor for carbocyclic nucleoside, was synthesized by cyclising the allylic alcohol **42**, through RCM protocol.³⁴⁶

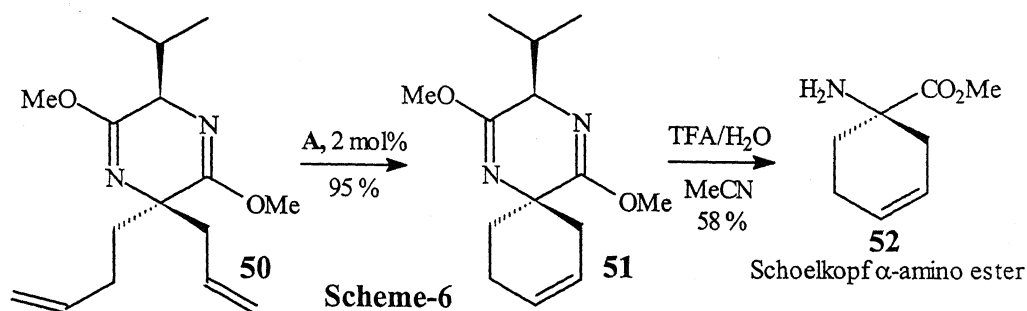


Cyclization of mono gem-substituted dienes **44** afforded trisubstituted cyclic alkenes, using **A**. But cyclization to the disubstituted cyclic olefin required **B**, and could not be synthesized with **A**.

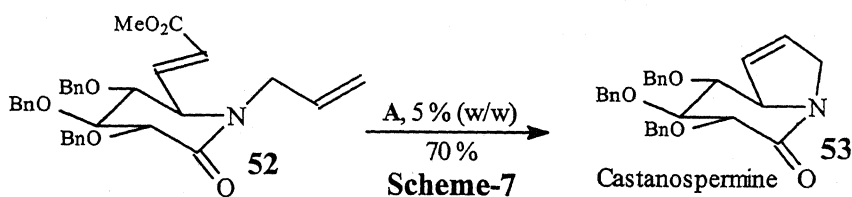


Monosulfide containing dienes **48** were cyclized in excellent yields to **49** with catalyst **B**. However, the yields for the formation of the cyclic olefins were strongly influenced by the substitution pattern around the double bonds.³⁴⁷⁻³⁴⁸

The Schoellkopf aminoacid synthesis followed by RCM gives, via pirocyclic systems, conformationally restricted α -amino esters which would otherwise be difficult to prepare in optically active form.³⁴⁹

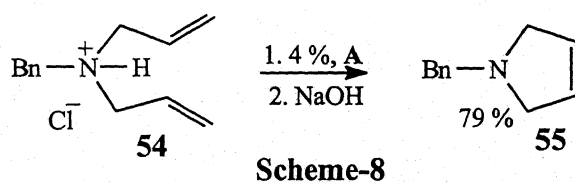


RCM has been employed as a key step in the synthesis of a natural product castanospermine,³⁵⁰ by a novel approach using a substrate with one of the olefinic groups as an α,β -unsaturated ester.³⁵¹



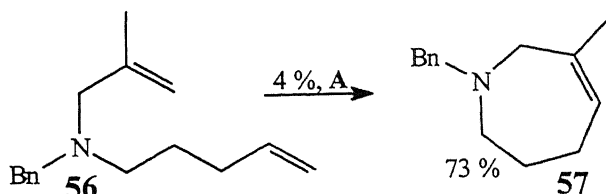
Application to Peptide Systems-

Due to the high activity and functional group tolerance of the metathesis catalysts A-C, RCM has recently extended its utility to the area of peptide chemistry. Except for in some cases, the RCM of NH amide containing diolefins is generally clean. The two most obvious causes of worry during the RCM of peptide substrates is the presence of NH and C=O functional groups. RCM reactions with basic amines is problematic because the catalyst gets inhibited by the complex-forming properties of amines and amides,³⁵² even with the use of Grubb's ruthenium catalyst.

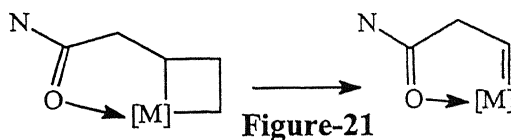


Benzyl diallylamine could be cyclized only after conversion into the hydrochloride **54**. However, the Schrock's catalyst seems to be better choice for this reaction.³⁵³ The

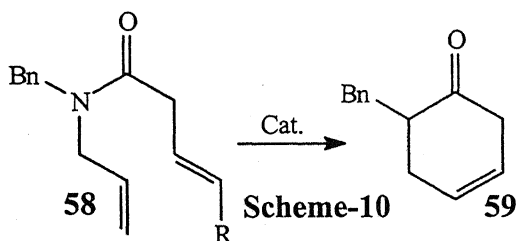
catalyst-inhibiting complexation with basic nitrogen can often be suppressed by steric hindrance.



In reactions with α,β - or β,γ -unsaturated compounds, the catalysts may become inhibited by intra-molecular interaction with the carbonyl group.



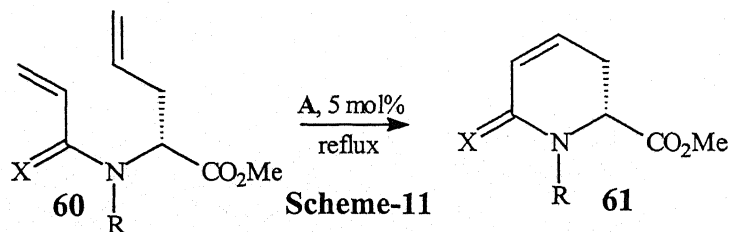
Use of higher substituted double bonds solves this problem (Grubbs³⁵⁴). Primary attack at the N-allyl group prevents formation of an inhibiting chelating structure.



| | | |
|--------|--------------|---------------|
| a = H | a, cat. = 3b | nocyclization |
| b = Et | b, cat. = 3b | 80 % yield |
| | a, cat. = 4b | 93 % yield |

Effects of complexing structural elem

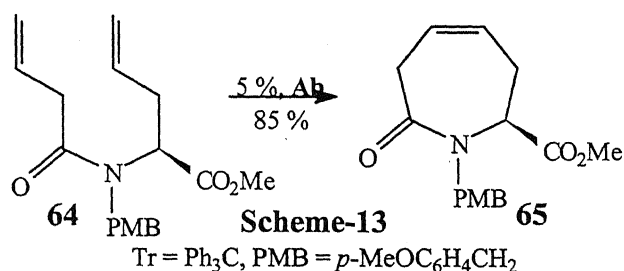
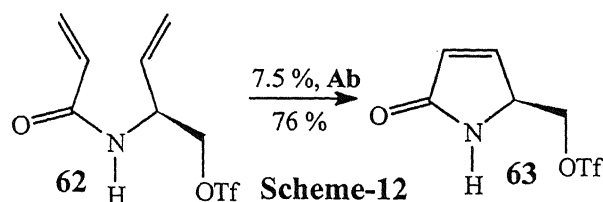
Thus, highly functionalised six- and seven-membered amino esters or acrylic amides **61** were prepared from aminoacid-derived dienes **60** with the Ru catalyst **A**.³⁵⁵



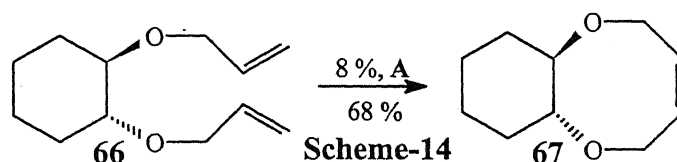
| | | | |
|-----------------------|------|----------------------------|------|
| X = O; R = H | 20 % | X = H ₂ ; R = H | 0 % |
| = PMB | 93 % | = PMB | 54 % |
| = CH ₂ Fer | 89 % | = CH ₂ Fer | 18 % |
| | | = Boc | 93 % |

In many cases Grubb's ruthenium catalysts are active even in the presence of free NH-amides. Starting with optically active vinyl and allyl glycine, various pyrrolidine and

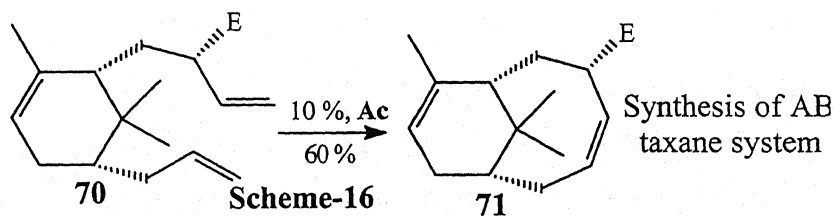
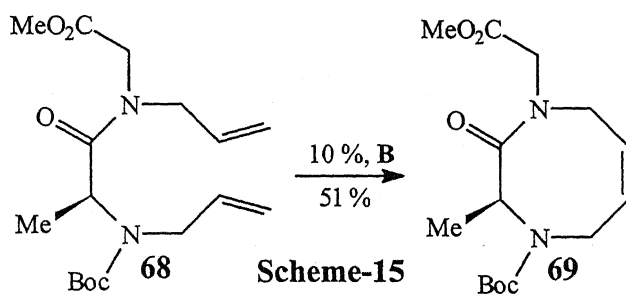
piperidine derivatives by RCM³⁵⁶ were synthesized. Although apparently favored due to steric blocking of the NH group by the trityl group, it seems that the NH-amides **62** and **64** could not be cleanly cyclized by catalyst **Ab**.



The balance between RCM and ROM (Ring Opening Metathesis) is affected by ring strain. Cyclization of diallyl ethers to eight-membered rings proceeded smoothly with Grubb's catalyst for the *Trans* compound than the *Cis* compound.³⁵⁷



Dipeptide **68** underwent a conformationally favoured cyclization to an eight-membered ring, the diazocine derivative **69** in 51% yield.³⁵⁸



The relatively strained AB-taxane system **71** was synthesized by RCM on the precursor **70**.³⁵⁹ (Scheme-16)

Cyclization to the nine-membered rings are more challenging than the eight-membered rings. Very less literature exists on the synthesis of nine-thirteen membered rings. Larger ring systems are generally more readily accessible. Due to the ring size, *cis* and *trans* double bonds can be formed. The yields are dependent on the ring size and the position of the resulting double bond.³³⁶

Although low yielding, the synthesis of the highly functionalized pentacycle **72**, containing the ABCD system of the alkaloid manzamine A is remarkable.³⁶⁰

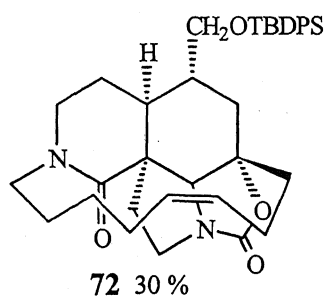
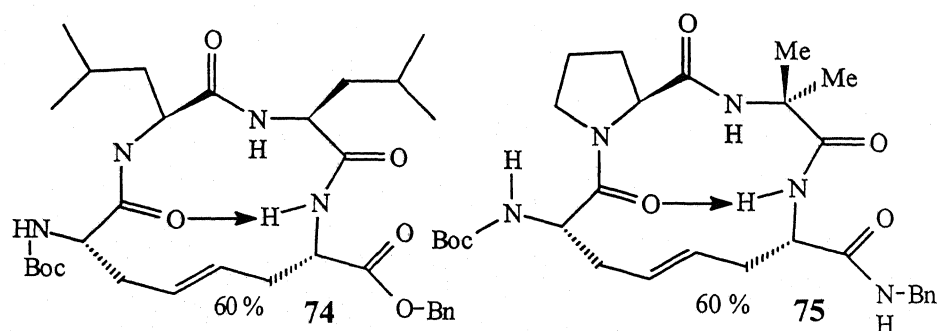


Figure-22 Manzamine

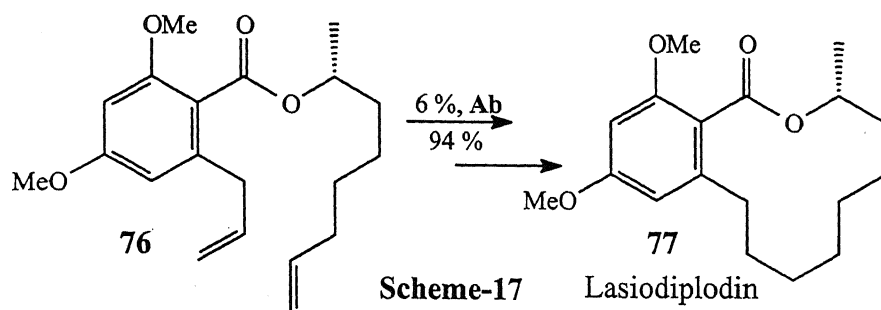
Another noteworthy example is the facile synthesis of peptides with and without conformational restrictions in the backbone.³⁶¹ Reaction of tetrapeptide precursor dienes with the catalyst **A** afforded 14-membered cyclic tetrapeptides **74**, **75**.



Scheme-23

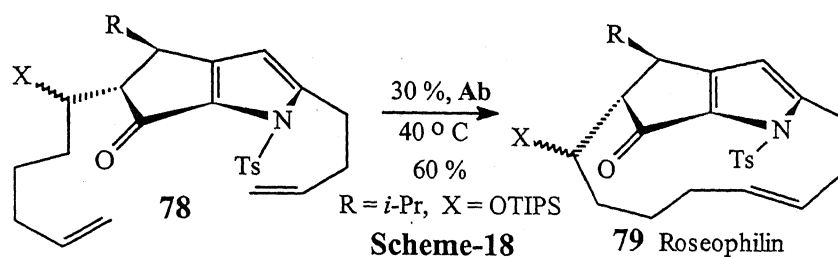
A 12-membered bicyclic macrolide Lasiodiplodin was synthesized quantitatively via RCM reaction by slowly combining two solutions of the diene **76** and the Ru-catalyst **Aa** via dropping funnels.³⁶²

The tricyclic core of roseophilin has been prepared using RCM as a key step.³⁶³ Cyclization of diene **78** required a bulky restraint group **R** on the bicyclic moiety and **X** on the olefinic handle, to provide the *ansa*-bridged silylether **79**.



Scheme-17

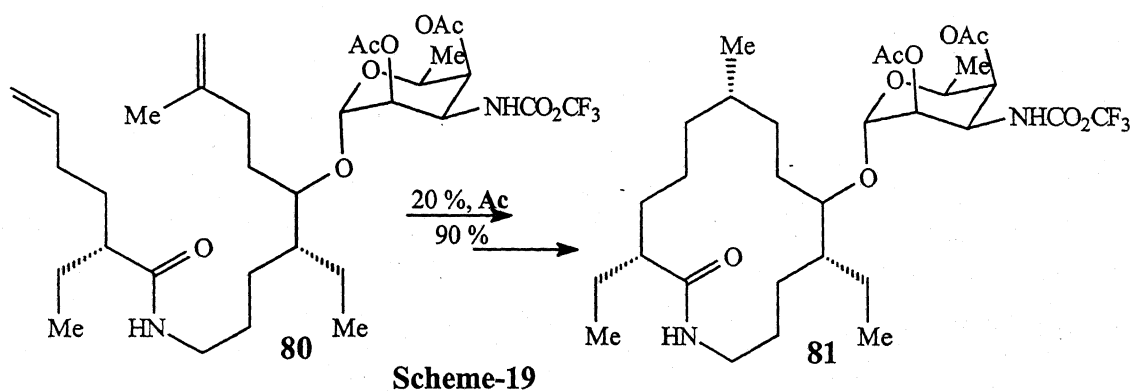
Lasiodiplodin



Scheme-18

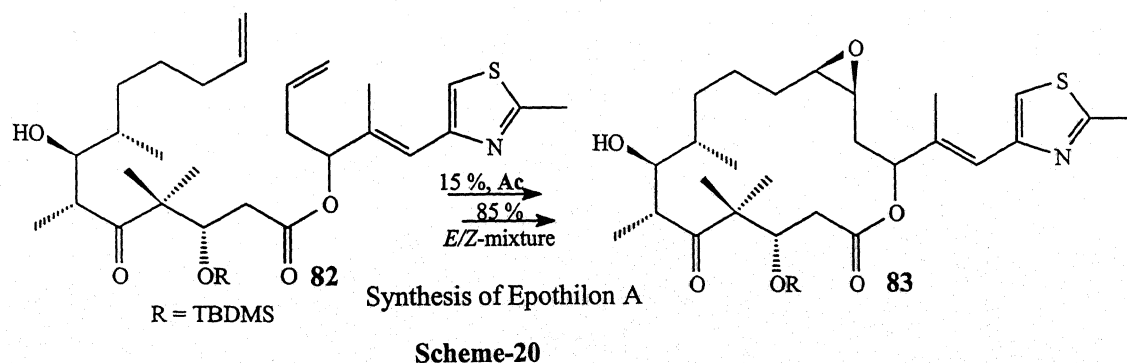
Roseophilin

Hoveyda *et al.* have reported the synthesis of the anti-fungal agent Sch38516 through the RCM of a fully functionalized acyclic precursor in excellent yield.³⁶⁴



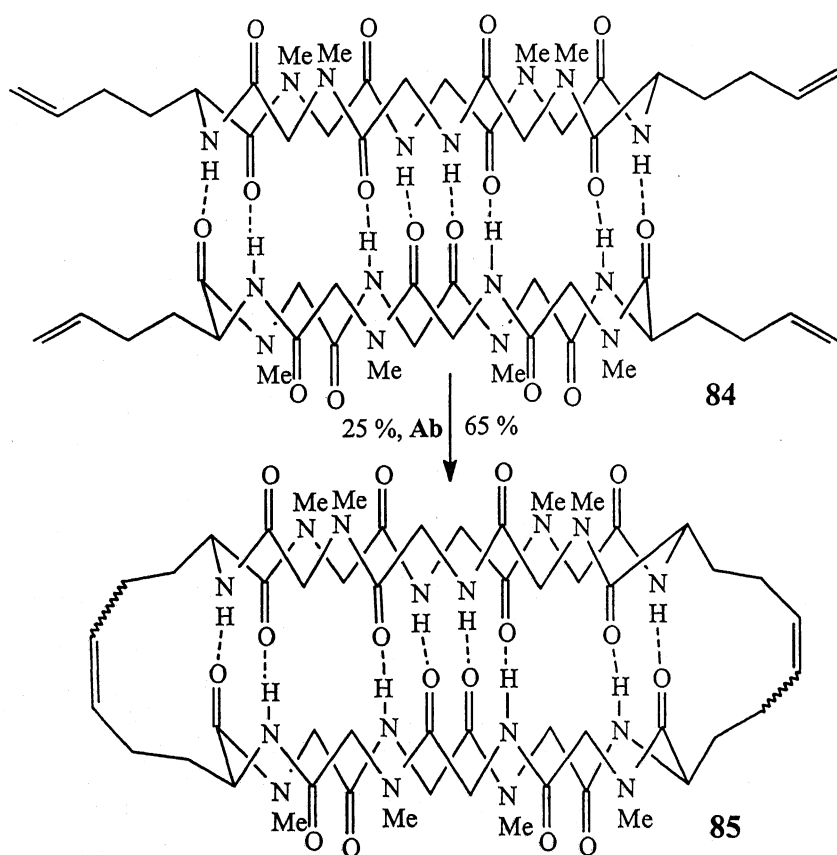
Scheme-19

Nicolaou's synthesis of Epothilin A is a classic culmination to the show of potential of the ring closing metathesis for the construction of complex molecules. Using RCM as the key step, Nicolaou *et al* synthesized Epothilin A from **82**, under mild conditions in 85% yield.³⁶⁵



Scheme-20

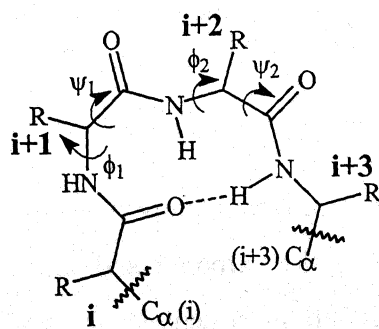
In the end, one of the largest cyclic compounds constructed by metathesis is the 38-membered ring **85**, by Ghadiri *et al*³⁶⁶ Supramolecular interactions between cyclopeptides **84** were used in the synthesis of this ring. Nonpolar solvents assist this cyclization by arranging the cyclic molecules to cylindrical arrays by intermolecular hydrogen bonding. The cyclization did not occur in polar solvents, due to lack of H-bonding.³³⁶



Scheme-21

Present Study

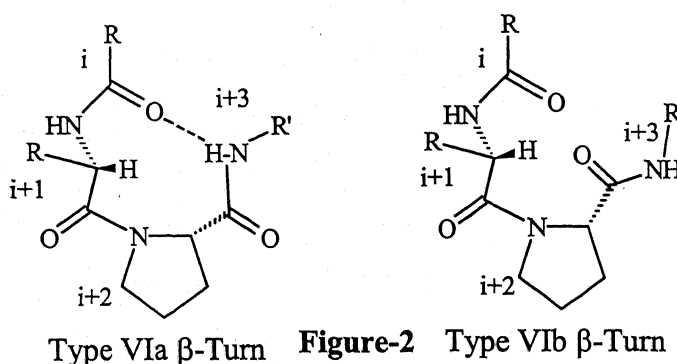
In light of the urgent need to counter the "silent epidemic", acquired immunodeficiency syndrome (AIDS) caused by a very adaptable and smart Human Immunodeficiency Virus (HIV), numerous drug design methodologies have evolved. The structural framework and reproductive mechanism of the virus has been very well understood by now; but the viral mechanism that results in its synthesis of resistant strains to newer drugs, is not. Libraries of small peptides that mimic the substrates are being designed and synthesized to arrive at a better understanding of the virus and its mechanism of action. Linear peptides are flexible and exhibit numerous conformations in solution and even in the solid state, which does not serve our purpose for better understanding of the enzyme activity. However, analogues of native peptide can be conformationally restricted in which important recognition elements are appropriately placed in a three-dimensional geometry. This would lead to better entropic binding with the active site and provide direct information about the bioactive conformation of the peptide ligand,³⁶⁷ and a great deal of indirect information about the three dimensional interplay between receptor and ligand, which would help us in designing better drug candidates. Frequently, such restrictions also result in increased affinity for a particular receptor with simultaneously diminished sensitivity to other peptidases resulting in greater specificity towards *that* receptor. Constraining peptides also result in better oral- and bio-availability of the ligands leading to their therapeutic utility as drugs. A number of X-ray structures of proteases with their corresponding peptide inhibitors show that local regions of peptides bound to active site adopt an extended conformation very similar to a protein beta sheet or beta strand. One important structural feature of many biologically active peptides and proteins is the β turn motif.³⁶⁸



The structure of a β -turn

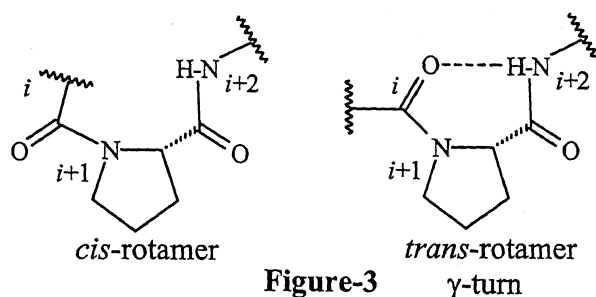
Figure-1

β -turn renders a local constrain with in the peptide framework through possible hydrogen bonding interactions. β -turn is a tetra-peptide sequence in which the $\alpha C_{(1)} - \alpha C_{(4)}$ distance is ~ 7 Å and which occurs in the non-helical region of a protein³⁶⁸. Several types of beta turns are found in proteins and the type VI turn is a unique member of the beta turn family because it is the only turn that involves an S-cis peptide bond. Type VI beta turns always contain a proline residue at the $i+2$ position since, peptides that incorporate this amino acid are the only ones that can exist substantially in the S-cis configuration. These are often found in peptides and proteins containing the sequence ArProAr, where Ar represents an amino acid with aryl side chain. Type VI β turns are subdivided into type VIa and VIb turns. In type VIa turns, an intramolecular hydrogen bond is formed between the carbonyl oxygen of the i^{th} residue and amide hydrogen of the $i+3^{\text{th}}$ residue. In the type VI β turns, this hydrogen bonding interaction is lacking because, the carbonyl C=O of the proline residue faces the carbonyl of the i^{th} residue, hence forcing the C-N amide bond of the $i+3^{\text{th}}$ residue away from the latter.



The understanding of the conformation of type VI beta turn is very crucial to the development of inhibitors for HIV protease. This is mainly due to the unique specificity shown by the HIV PR, unlike other mammalian endopeptidases, for the selective cleavage of Phe/Tyr-Pro amide bonds in the Matrix-Capsid domain of the gag-pol polyproteins. Hence, incorporation of this type of beta turns in native peptide ligands could lead to biologically active peptides.

The *trans* rotamer of the L-proline containing peptides can also adopt the γ -turn due to the appropriate positioning of the carbonyl and the amide NH groups for hydrogen bonding. Gamma turn is found in tripeptide sequences. It is also identified as an important structural element for imparting constraints in conformations of peptides.



In view of the importance of constrained conformations, there have been several attempts to lock peptides into turn configurations and to synthesize molecules that might mimic a turn in an otherwise normal peptide. However, we wished to *utilize* this turn among proline containing peptides for the synthesis of conformationally constrained small peptides incorporating the hydroxymethylcarbonyl (HMC) isostere, as structure and mechanism based inhibitors of HIV PR.

Given such a background, our objective was to synthesize and conformationally constrain acyclic peptides incorporating the unnatural aminoacid statine analogue (AHPPRA) β -phenylisoserine, containing the hydroxymethylene isostere (HMC), into cyclic peptides. We envisioned that the tripeptides derived from sequence where L-proline is involved in a gamma turn would be an ideal precursor for such cyclisations.

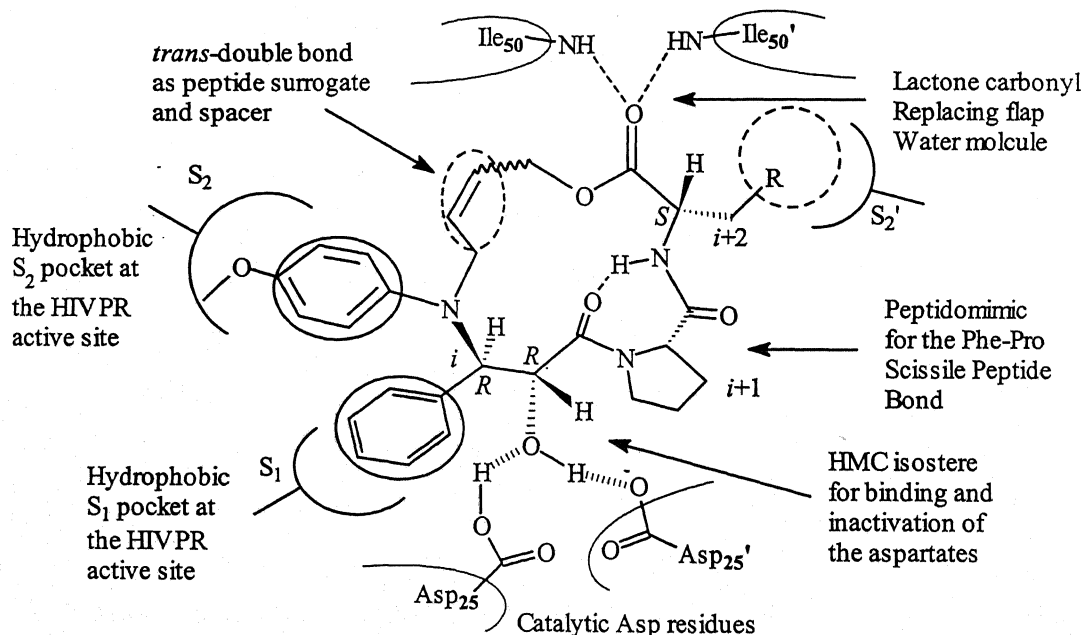
In view of this goal, we set to synthesize small peptides (di- and tri-peptides), incorporating the β -phenyl isoserine unnatural aminoacid, containing functional groups that can be manipulated by suitable chemical transformations to the corresponding conformationally constrained cyclic peptides. The highly functional-group friendly, transition metal catalysed ring closure metathesis (RCM) protocol was the chemical transformation of choice to both cyclise and to generate another peptide surrogate as a spacer for cyclisation, the *trans*-double bond.

We reasoned that cyclic tripeptides of the type A where proline is incorporated in the $i+1$ / $i+2$ position, would serve to be good leads as inhibitors of HIV-PR based on the following considerations about the known recognition and structural elements in the HIV PR active site.

1) Presence of proline residue in the tripeptide is essential, due to its unique recognition by the HIV PR; it imparts a turn (β/γ -turn) to the peptide at any position and hence structurally preorganises the peptide for cyclisation; presence of the turn motifs would prove to be advantageous to arrive closer to the biological conformation of the peptide.

- 2) The lactone carbonyl, as amply demonstrated ^{241,369} in earlier parts of the review, could serve to replace the structural flap molecule at the HIV PR active site by forming hydrogen bonds with the flap Ile NHs, an entropically and enthalpically favourable process.
- 3) The HMC isostere would, as demonstrated in the previous sections, serve as a peptidomimic and bind to the active aspartyl groups as a surrogate of the structural water molecule
- 4) The aromatic groups would provide the required hydrophobicity to the P₁ site and even extend to the P₂ site, a characteristic requirement of HIV PR active site pockets
- 5) Appropriate amino acid residues at P₁' and P₂' for better binding.

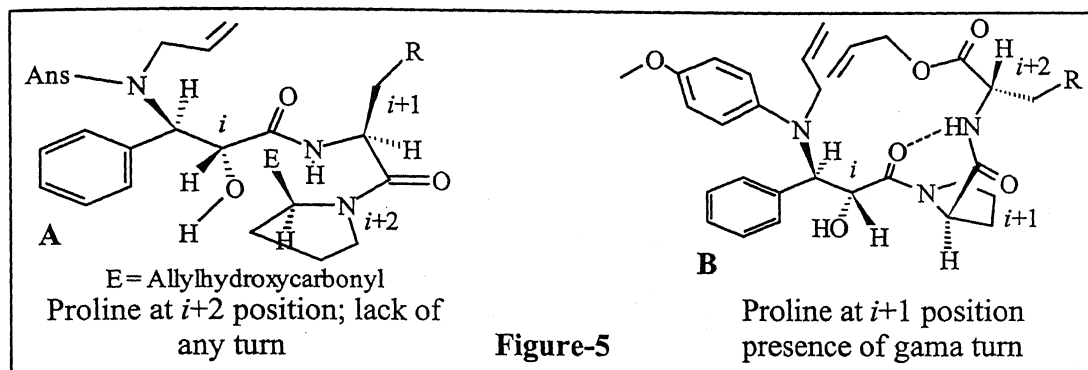
We reasoned that cyclisation would be eased by placing a proline residue at *i*+1 position, which would serve to both induce a turn, and to mimic the Phe-Pro or Tyr-Pro scissile bond of the HIV PR substrate cleavage sites.



Schematic representation of the Proposed Positive Ligand-Enzyme Binding interactions of the cyclic tripeptides 1, 2 and HIV PR

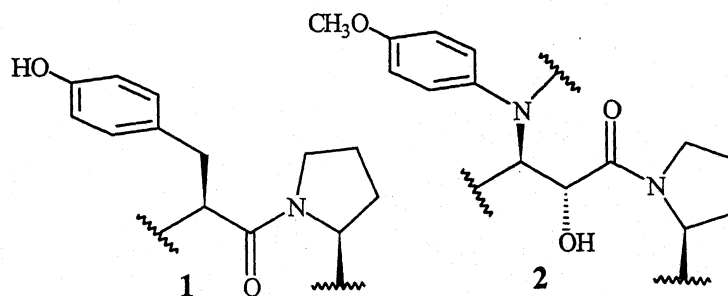
Figure-4

How crucial is this turn for conformationally constraining the peptide, would be best understood by disallowing a beta turn, with proline as the *i*+2 residue (Figure-5) and the absence of a fourth peptide which precludes the possibility of any turn. Keeping in consideration, the peptide bonds, aromatic π -stacking interactions (between the phenyl and aryl ring systems), γ -turn and the presence of a cyclic amino acid residue (proline) as



part of the cyclic peptide, the resulting macrocyclic 15-membered rings would be restricted to a narrow degree of rotational and torsional freedom which is a desired feature. The *trans* double bond would act as a suitable spacer amidst all these restrictions as well as serve as a peptide surrogate.

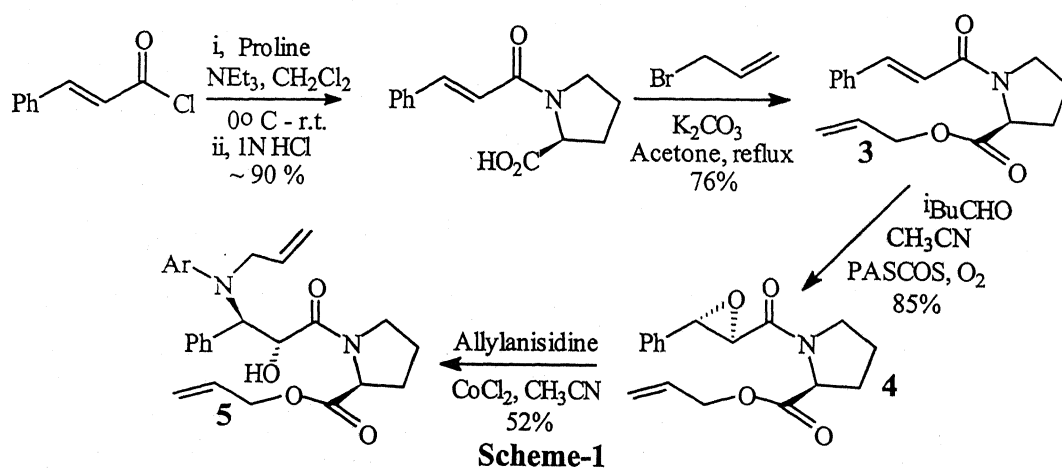
These peptides, incorporating a β -phenylisoserine derivative, can be synthesized with the help of the PASCOS catalyzed methodology, as described earlier. Epoxidation of the N-cinnamoyl-dipeptide-allylesters and its opening with a suitable aromatic amine, containing a "N-terminal handle" for later cyclisation, would lead to the synthesis of tripeptides of the type **A** / **B**. Allylanisidine and allyl ester were chosen as the N- and C-terminal handles respectively. Allylanisidine was chosen for the following reasons. (i) it would mimic the Tyr-Pro scissile amide bond at HIV PR substrate cleavage sites (Figure-6); (ii) RCM catalysts need amines to be sterically protected as tertiary amines, in the substrate, for efficiency in reactions and (iii) the stereochemistry of ring opening of epoxides by secondary-anilines has been established by us as "anti".³⁷⁰



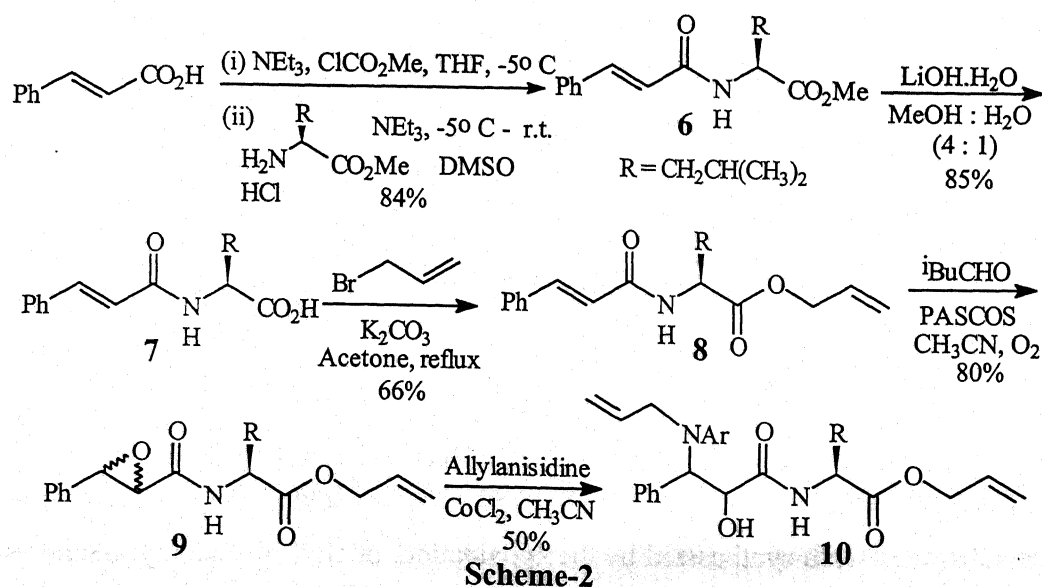
Allylanisidine handle in **2**, mimicing the Tyr- residue of Tyr-Pro peptide bond-1

The substrates were synthesized by the epoxidation, of allyl-N-cinamoyl-amino esters in the presence of polyaniline supported cobalt (II) salen, and the subsequent opening of the epoxide by allyl anisidine, mediated by cobalt chloride.

We conducted our initial studies with the structurally simple dipeptides incorporating the β -phenylisoserine derivative, containing the N- and C-terminal olefinic handles. Thus O-allyl-N-cinnamoyl-(L)-proline **3** and O-allyl-N-cinnamoyl-(L)-leucinate **8** were synthesized from their corresponding carboxylic acids by esterification with allyl bromide in acetone. N-cinnamoyl protected proline was synthesized directly by coupling equivalent amounts of cinnamoyl chloride with proline in the presence of triethylamine, in either dichloromethane or acetonitrile, at 0 °C.



Whereas, N-cinnamoyl-(L)-leucine was synthesized by base hydrolysis and subsequent acidification of the corresponding methyl ester, obtained by mixed anhydride coupling of cinnamic acid and methyl-(L)-leucinate hydrochloride.



These diolefinic substrates were subjected to aerobic oxidation in the presence of polyaniline supported cobalt (II) Salen. It was gratifying to note that chemoselective epoxidation occurred, exclusively on the cinnamoyl double bond, in excellent yields.

While facial selectivity was observed ($[\alpha]_D^{CHCl_3} = -64.28^\circ$; $c = 0.007$) in the case of proline containing dipeptide isostere **4**; low rotation value for **9** ($[\alpha]_D^{CHCl_3} = +8^\circ$; $c = 0.005$) implied poor facial selectivity. Epoxide **4** was obtained as a mixture of two diastereomers (1:1.8; from 1H NMR). We assigned the stereochemistry of the major diastereomer to be (3*S*,2*R*), from the negative rotation value of **4** (correlation from the corresponding methylester). These epoxides were opened with allylanisidine catalysed by cobalt (II) chloride to obtain the β -phenylisoserine containing dipeptides **5** and **10** in good yields. Dipeptide **4** yielded the *anti*-diastereomer of **5** predominantly (1:2.2). Cobalt (II) chloride was chosen over the polymer-supported catalyst for opening because surprisingly, we observed better yields for opening of epoxides of allyl-*N*-cinnamoyl-amino esters, with allylanisidine by the former over the latter.

For the RCM on these substrates, we chose the relatively more stable and more reactive of the metal alkylidene catalysts, the Grubb's ruthenium benzylidene catalyst.

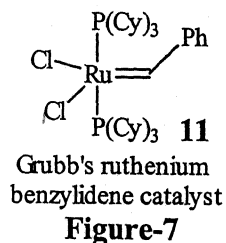
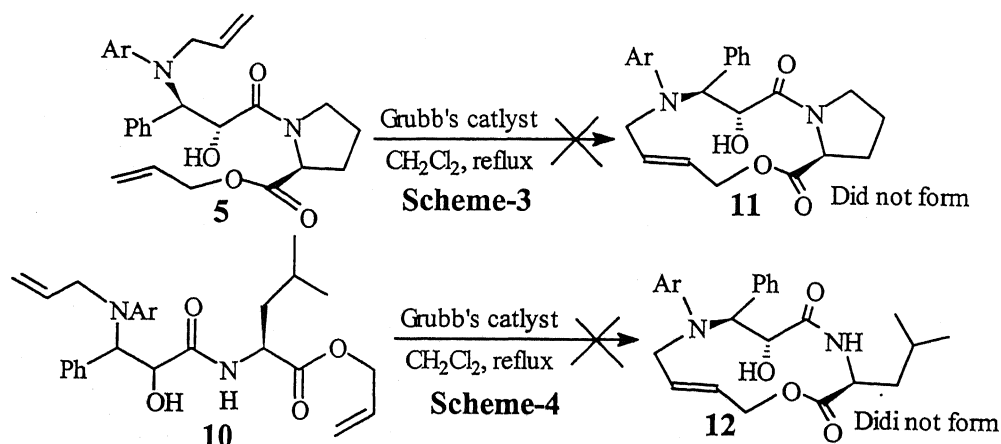


Figure-7

With the absence of any structural pre-organising elements like the β - or γ -turn, and although too less literature exists for the synthesis of 10-14 membered cyclic compounds using the Grubbs catalyst, we expected better cyclization in the case of **5** than **10**. We reasoned the possibility of an 180° turn provided by the proline residue, if at all, to bring the terminal olefins closer for cyclization.

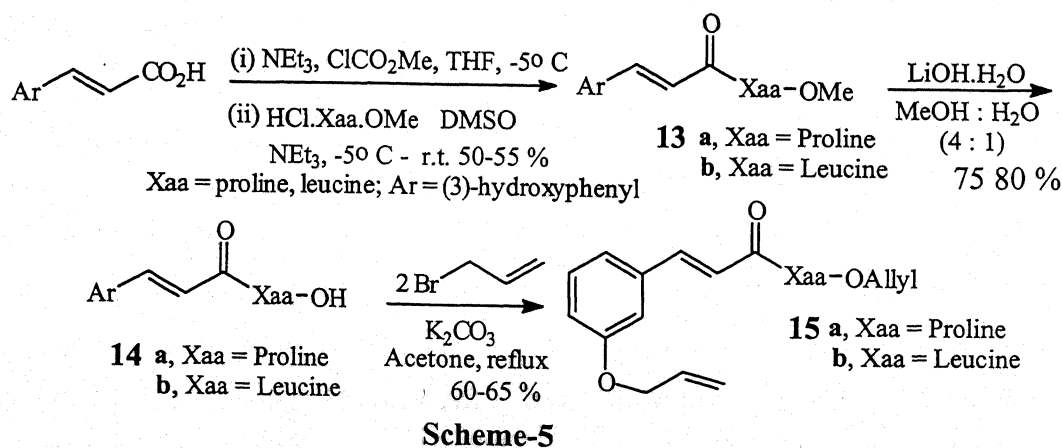
Typically, Grubb's catalyst **11** (10 mol% of diene) was taken in a dry flask and flushed with a constant flow of nitrogen. To this was added, dry CH_2Cl_2 (0.012 mM with respect to the amount of diene) and stirred vigorously for 10 min. A violet coloured solution resulted. A solution of diene in dry CH_2Cl_2 (0.012 mM) was added slowly (~ 2 mL/min) to the stirring solution. After complete addition, the reaction mixture was set to reflux under nitrogen atmosphere for 18 h. Then, a fresh lot of a solution of **11** (10 mol%) in CH_2Cl_2 was added to the reaction mixture and stirring continued for another 18 h, after which, the solvent was removed and the resulting residue, purified by column chromatography. But **5** failed to cyclise in the presence of Grubb's Ruthenium alkylidene catalyst under several conditions of high dilution, catalyst concentrations and longevity

of the reaction. The complete recovery of the starting material led us to believe that the 12-membered ring was too strained to form due to the presence of cyclic proline residue. However, subjection of **2** which lacks such strain, to Grubb's cyclization conditions also did not yield any positive results.

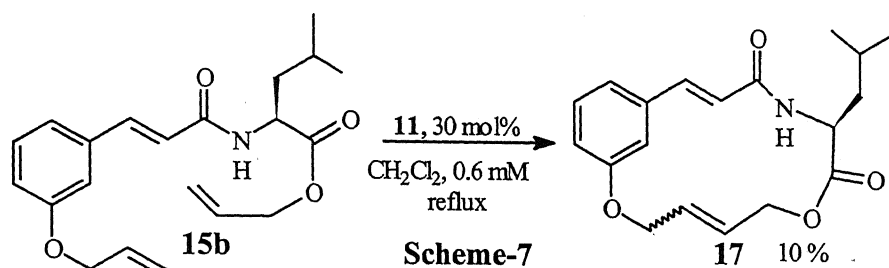
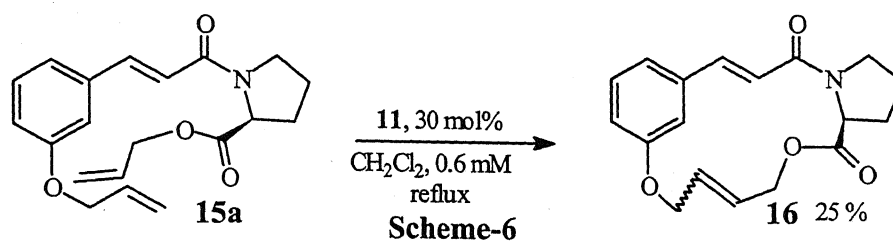


However, to check the possibility of the catalyst being "killed" by chelation with the nitrogen, we attempted the cyclisation of the non-amine containing dipeptides; allyl-N-(3'-allylhydroxycinnamoyl)-proline **15a** and -leucine **15b**. These were synthesized by the di-allylation of the corresponding hydroxy acids **14a,b** with allyl bromide in the presence of potassium carbonate.

The presence of a third olefinic double bond (the cinnamoyl double bond) apart from the terminal olefins in **15a** and **15b**, was seen as any problem in this reaction, as it would be much less reactive and strained to participate in RCM.



Subjection of **15a** & **15b** to Grubb's cyclisation conditions yielded the cyclised product in minor (25 % & 10 % respectively) amounts, among other oligomeric products and the recovered starting material.

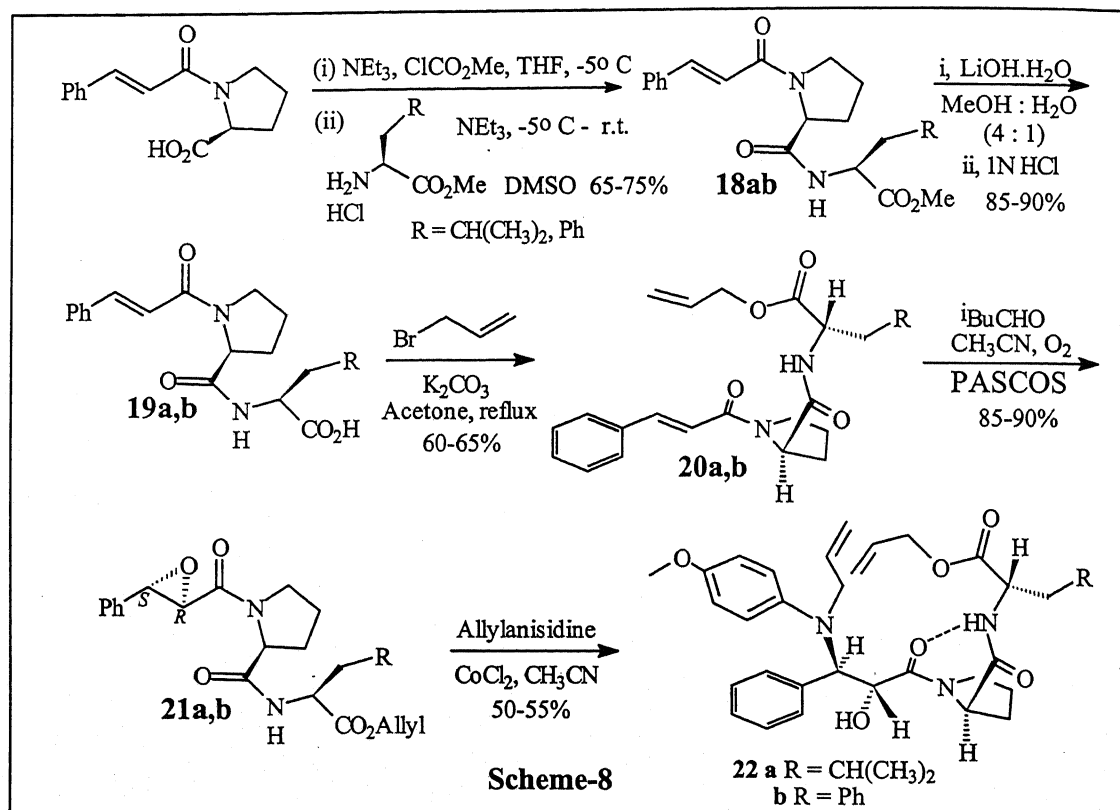


The low yields of the cyclised product suggested the presence of high-energy ring strain in these dipeptides. This ring strain could be responsible for the lowering in the rate of cyclisation, than the rate of decomposition of the catalyst - and hence resulting in recovery of the dienes in the former cases. However, we expected that unlike in the case of these pseudo-dipeptides, the corresponding tripeptides, where cyclisation would yield larger (15 membered) rings, this strain would be much less and the possibility of turn structures in the molecule would add to facilitate the cyclisations.

Thus, we cautiously turned our focus to the synthesis of cyclic tripeptides through their acyclic precursors. The olefinic tripeptides, for epoxidation were synthesized with proline as the $i+1^{\text{th}}$ and $i+2^{\text{th}}$ amino acid residue from the cinnamoyl group, by the mixed anhydride coupling procedure using methylchloroformate as the C-terminal activating group in THF (Scheme-8).

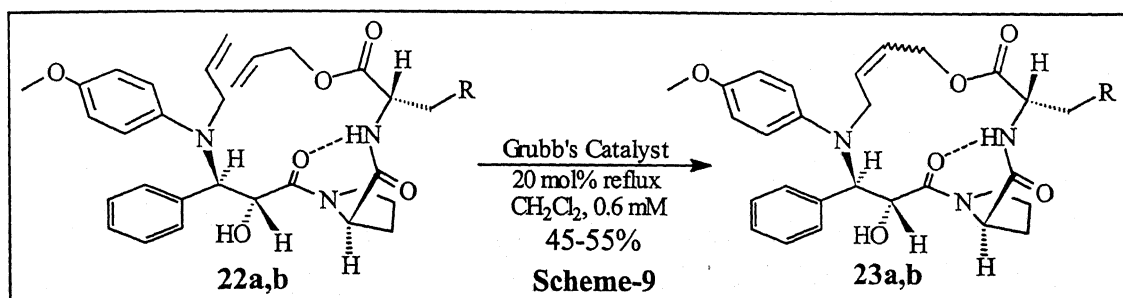
The syntheses of N-cinnamoylproline containing peptides were higher yielding and less cumbersome. These were synthesized as shown in Scheme-8. N-protection of proline was accomplished in dichloromethane or acetonitrile by the slow addition of one equivalent of cinnamoylchloride to a suspension of proline in a solution of triethylamine in the solvent at $0\text{ }^{\circ}\text{C}$ and stirring for 7-8 hours. Washing with acid (1N.HCl) and drying yielded the N-cinnamoyl-proline, which was coupled with the second amino acids by the mixed anhydride coupling procedure. To a solution of N-cinnamoyl-proline in THF was added triethylamine, at $-5\text{ }^{\circ}\text{C}$. To this was added methylchloroformate followed by a solution of the methyl-amino ester hydrochloride in $(\text{CH}_3\text{O})_2\text{SO}$, and triethylamine. Base workup of the concentrated reaction mixture followed by its purification in column

chromatography gave the N-cinnamoyl-proline containing dipeptides, **20a,b**. Base hydrolysis of the N-cinnamoyl dipeptide esters and its subsequent acidification with acid, yielded the corresponding carboxylic acids, which were esterified with allylbromide in the presence of K_2CO_3 in acetone (Scheme-8).



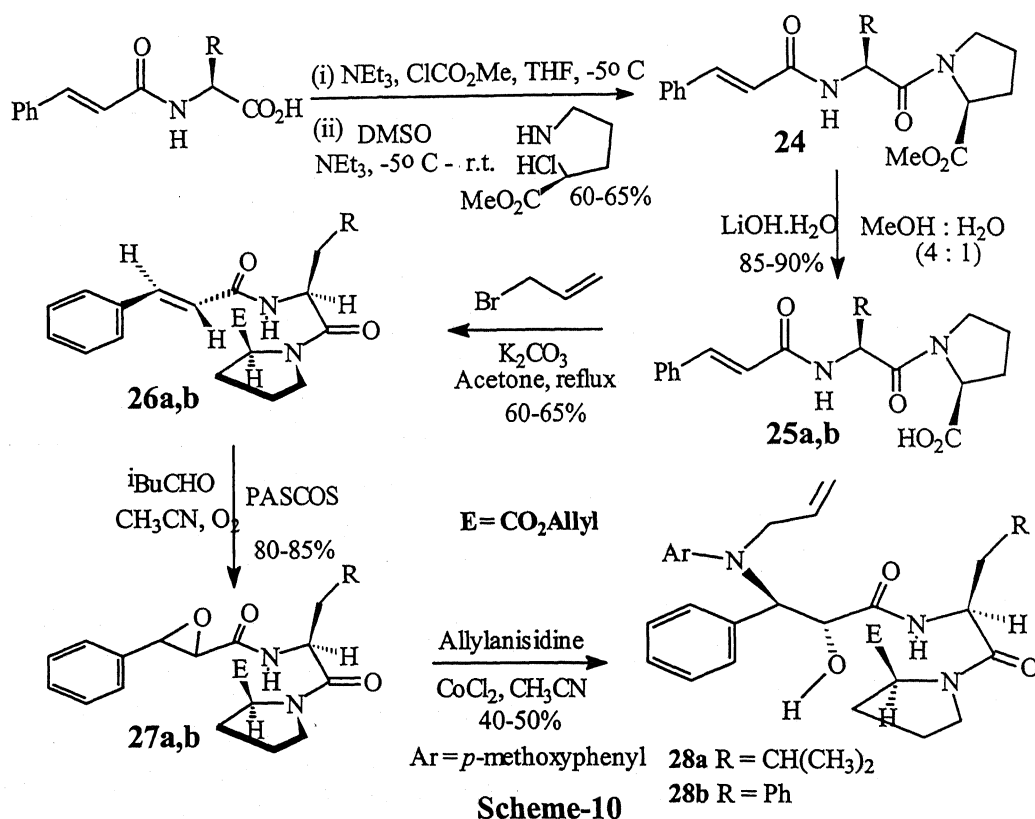
The tripeptide derivatives **22a,b** were prepared by our polyaniline supported cobalt (II) salen (PASCOS) catalyzed aerobic epoxidation and its opening with allylanisidine, as described in Scheme-8, from the peptide precursors. The peptides **20a** and **20b** were subjected to aerobic epoxidation in the presence of 2-methylpropanal and catalytic amounts of PASCOS to yield the corresponding epoxides **21a** and **21b** respectively as predominantly one diastereomer. From sign of rotation (negative) and correlation with the 1H NMR of the precursor methylester, the absolute stereo-chemistry of the predominant (7:3 (2.5:1) diastereomer was assigned as (3S,2R) – from the cinnamoyl carbonyl (ratio obtained from the ratio of 1H NMR signals for the β -epoxymethine proton - verified by correlation with the studies on the epoxidation of Methyl-N-cinnamoyl-(L)-proline-(L)-leucinate). Examination of the N-cinnamoyl-dipeptide-allylesters for their low energy conformations with the help of Hyperchem energy minimization package showed that, in **20a,b**, the allyl groups provided greater facial bias

to the cinnamoyl double bond, than rendered by their methyl ester counterparts, hence resulting in the observed increase in diastereoselectivities. We have shown earlier that the cobalt catalyzed opening of cinnamoyl epoxides take place by an S_N2 pathway leading to the *trans* distereomer as the predominant product. Thus we obtained two distereomers each, for **22a** and **22b**. The predominant one in each case (3:1) was seen to possess the *trans* stereochemistry. These peptides showed the presence of intramolecular hydrogen bonding as indicated by the appearance of low field ^1H NMR signal at 7.43 ppm ($J = 8\text{Hz}$) due to amide proton. The presence of intramolecular hydrogen bonding in these structures suggested that these molecules are preorganized due to presence of γ -turn which may facilitate the cyclization of **22** via ring closure metathesis using Grubb's catalyst.³⁷¹ This indeed was the case; as subjecting **22a** and **22b** to ring closure metathetic conditions yielded the corresponding cyclized peptides in moderate yields (Scheme-9).



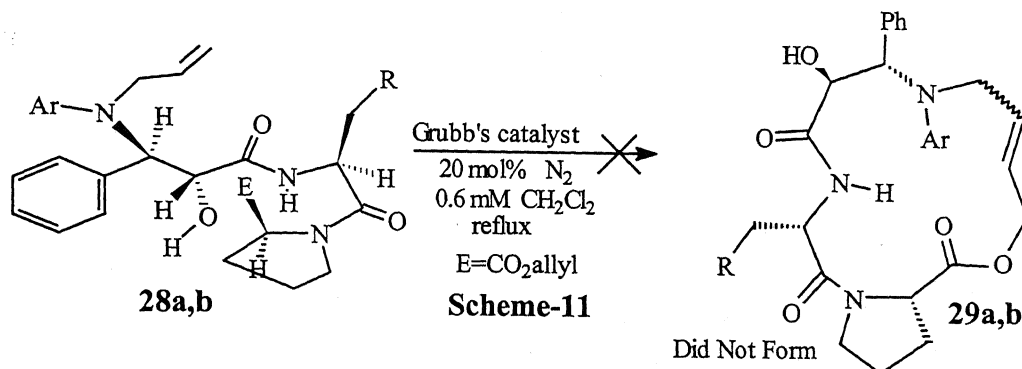
A solution of the diene (**22a** or **22b**) in dichloromethane (0.3mM) was added drop wise to a stirring solution of the Grubb's ruthenium benzyldiene catalyst (20mol%) in dichloromethane (0.3 mM with respect to the diene). After complete addition, the mixture was set to reflux under N_2 atmosphere for 36 hours, quenching the catalyst by exposure to air, concentration and purification by flash column chromatography, yielded the cyclized products **23a** and **23b** respectively from **22a** and **22b**, in 45-55% yield. These cyclic peptides were obtained as a mixture of E:Z (4:1) isomers as indicated by ^1H NMR. The reaction mixture also contained some oligomeric products and about 20% of the tripeptides **4** were recovered unchanged. By comparison of the ratios of the anisylmethoxy peaks for the two diastereomers in the recovered product, it was evident that the major *trans*-disatereomer had cyclized in about 60% yield. The proton NMR (7.45ppm, $J=8.23\text{Hz}$) of the cyclic peptides **6** revealed the presence of intramolecular hydrogen bonding which clearly suggests that γ -turn is also present in the cyclic form and may be responsible for the cyclization and the earlier observed diastereoselectivity.

That the presence of a γ -turn may be responsible for such cyclization is evident from the ring closure attempts on the acyclic tripeptides **28a** and **28b**, containing L-proline as the C-terminal residue (Scheme-10). The tripeptides **28a,b**, which lack a γ -turn, were synthesized by the protocol as defined in Scheme-10.



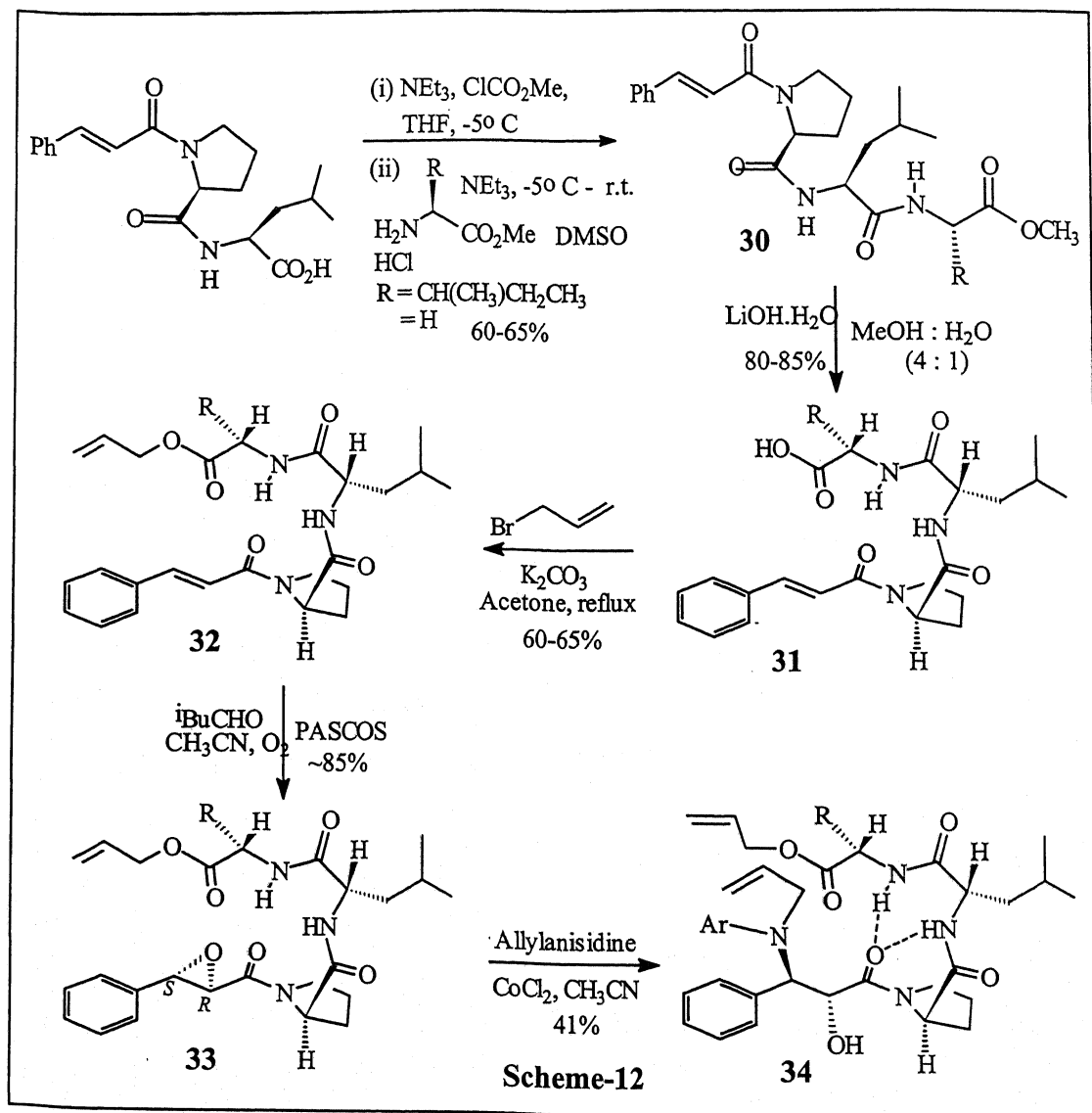
Thus, the methyl-N-cinnamoyl-dipeptide esters **24a,b** were converted to the corresponding allyl-N-cinnamoyl dipeptide esters **26a-b** respectively, by the mixed anhydride coupling method. These allyl ester dipeptides were transformed to the corresponding epoxides **27a,b**. The epoxidation was effected in the presence of 3-methylpropanal and catalytic amounts of PASCOS, under aerobic conditions. These epoxides also showed a negative sign of rotation and thus it was concluded that the (3S,2R) diastereomer was formed predominantly (1.6:1-62:38) (^1H NMR) in these cases too. These epoxides were subjected to cobalt (II) chloride catalysed opening by N-allyl anisidine as described earlier to afford the tripeptides **28a**, **28b** respectively. The tripeptides **28a** and **28b** were subjected to ring closure metathesis reaction in dichloromethane (0.6 mM) using the Grubb's catalyst (20%). The reaction was performed at higher dilution (0.1 mM) and concentration (3mM), for longer periods in the presence of, upto 30 mol% of Grubb's catalyst, but to no avail.

To our initial dismay, but later interestingly, the cyclic peptides **29a** or **29b** were not observed and the reaction mixture consisted of intractable oligomeric material apart from the unreacted **28a,b** (~20-30%). Among other non-polar components of the mixture was the notable presence of tricyclohexyl phosphine. It was studied by Grubb's *et al* that the Ruthenium catalyst gets decomposed in the presence of sterically unencumbered amines, due to ligation with the amine.



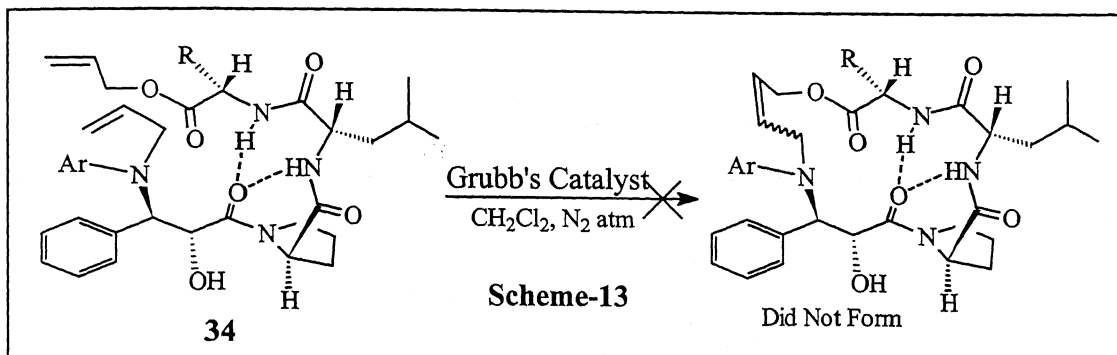
That a cross metathesis of the metal alkylidene complex (formed with the terminal olefin) can occur with the carbonyls in the substrate, has been reported in many cases. This is especially true for systems with amide groups having free N-H. And, hence we concluded that the observed intractable side-products were due to these side reactions during the ring closure metathesis of the tripeptides **28a-b**. It is interesting to note the absence of such an observation in the case of **23a-b**. Thus, proline as the (i+1) residue in these tripeptides, seems to be essential to; i) provide a γ -turn to the tripeptide for its structural pre-organization, whose absence in **28a-b**, did not yield the desired cyclic product and ii) render the carbonyl unavailable for any undesired side reactions. This was also substantiated by molecular model studies on these RCM precursors. Energy minimization studies on the RCM precursors **22a,b** showed the unambiguous presence of a g -turn motif, due to the presence of proline. The energy minimized conformation of the RCM precursors **22a** and **22b** showed that the terminal olefins get placed in a converged fashion, for facile cyclisation in the presence of a carbene initiator (**11**) (refer to the molecular model diagrams). In the case of **22a**, the energy barrier to be overcome, for the two olefins to react is only the 180° flip of the isobutyl side chain. In the case of **22b**, the barrier is even lesser. The energy minimization studies on the cyclised products **23a,b** revealed that the constrained peptides containing the *E*-double bond was strain free and of lesser energy, than that comprising the *Z*-double bond.

We wished to synthesize bigger macrocycles, by going from tri- to tetra-peptide with the proline as the $i+1$ residue. Since N-cinnamoyl proline leucine system was the most successful and high yielding substrate among the tripeptides, we chose to extend the tripeptide with isoleucine due to its relative abundance in many cleavage site sequences of substrates, by HIV PR.



Thus, the N-cinnamoyl tripeptide was synthesized as shown in Scheme-12. Amino acid residues were added to the peptide chain, using the mixed anhydride method. Base hydrolysis and O-allylation with allyl bromide, in acetone, of the methyl ester **30**, gave the corresponding allylester **32** in good yields. The N-cinnamoyl-tripeptide **32** was subjected to epoxidation conditions in the presence of PASCOS and 2-methylpropanal, under aerobic atmosphere resulting in the epoxide **33** in good yields. The epoxidation proceeded highly diastereoselectively and the absolute stereochemistry of the major

diastereomer (\sim 9:1, ^1H NMR) was assigned as (3S,2R) from the negative sign of rotation of the epoxide and its ^1H NMR correlations. Opening of the epoxide **33** in the presence of cobalt chloride with allylanisidine gave the β -phenylisoserine containing tetrapeptide **34** in good yields, predominantly (3:1) as the *anti* diastereomer.



The tetrapeptide **34** was subjected to RCM, in the presence of 20 mol% of Grubb's catalyst in dichloromethane (0.6 mM) in reflux conditions as described earlier. In spite of the presence of structural preorganization in the opened product-tetrapeptide, as shown by the low field ^1H NMR signal for the amide protons at δ 6.93 and δ 7.18 ppm the reaction did not proceed as expected. The starting material was recovered (45 %) along with many intractable high polar products. Probably, with the choice of allylanisidine and allylester as the diene handles, it seemed from these experiments that tripeptides were best suited for cyclisation through the RCM protocol using Grubb's ruthenium alkylidene catalyst **11**. The increase or decrease in chain length of the tripeptide, incorporating the β -phenylisoserine moiety, seems to kill the catalyst or result in oligomeric products.

Thus we have demonstrated the chemo- and substrate-directed stereo-selective epoxidation of several N-cinnamoyl peptides. Stereoselectivity seems to increase with increase in chain length. Peptides with proline residue alpha to the cinnamoyl olefin undergo epoxidation with greater stereoselectivity reaffirming the presence of additional secondary structural elements like γ,β turn in the peptides, as observed in ^1H NMR. Different di-, tri- and tetra- peptides, incorporating the β -phenylisoserine moiety containing the HMC isostere were synthesized. These are proposed to be prospective leads as drug candidates of HIV PR inhibitors.

Cyclisation studies on the acyclic dienes using the RCM protocol with the help of Grubb's ruthenium benzylidene catalyst showed need for a preorganisational element in the peptides for cyclization. Proline at the $i+1$ position of the peptide, provided the

required γ -turn to the molecule, thus structurally preorganizing the terminal olefins for metathesis. In larger peptides, there seems to be a need to alkylate the amide nitrogens, as they may hinder the activity of the catalyst. We have reported the cyclization of two of the tripeptide precursors to get to novel cyclic peptides which are proposed to be prospective drug leads in AIDS therapy as HIV-1 PR inhibitors. These cyclic structures contain the required binding elements *viz. a viz.*, the lactone carbonyl, to displace the flap water in the HIV PR active site for greater entropic binding; the hydroxymethylcarbonyl isostere which is most essential for binding with the aspartyl groups, displacing the structural water molecule from the active site and hence the inactivation of the virus; and the presence of hydrophobic side chain elements at P_n-P_n' sites to meet the hydrophobic requirements at the S_n-S_n' pockets in the HIV PR enzyme.

Experimental Section

Materials and Methods

Acetonitrile, ethyl acetate, hexane, THF and all other solvents were purified by standard procedures. CoCl_2 was purchased from LOBA Indian Limited and dried at 140°C for 4 h before use. All the amino acids were bought from SpectroChem India Limited and used as such. Cinnamic acid, anisidine, allyl bromide, triethyl amine, potassium carbonate, ethylene diamine, methylchloroformate, 2-methylpropanal (isobutyraldehyde) were all procured commercially and were purified before use. The secondary amine N-allyl-*p*-anisidine was synthesised following the known procedure.¹⁸¹ The aldehydes were distilled before use. Amines were re-crystallized before use. Polyaniline supported Co(II) Salen was prepared according to procedure developed in our lab.¹⁸² Column chromatography was performed on ACME silica-gel eluant. TLC was performed on ACME silica-gel-G coated glass plates and were visualised using UV lamp. ^1H NMR spectra were recorded using Jeol PMX-60 system, Bruker WP-80, Jeol 300 FTNMR or JNM-LA400 FTNMR machines in $\text{CCl}_4/\text{CDCl}_3$. Chemical shift are given relative to TMS in ppm(δ). Multiplicity is indicated using the following abbreviations: s(singlet), bs(broad singlet), d(doublet), dd(doublet of a doublet), ddd(doublet of a doublet of a doublet), dt(doublet of a triplet) td(triplet of a doublet), q(quartet) and m(multiplet). The FAB mass spectra were recorded on JEOL SX 102/DA 6000 mass spectrometer data system using Argon (6 Kv, 10 mA) as the FAB gas. Optical rotations were measured in Autopol[®] II/ Autopol[®] III polarimeters. All the known compounds were characterized by comparing with the literature data. IR spectra were recorded on Perkin Elmer 683 spectrophotometer, using either a neat sample or a solution in $\text{CCl}_4/\text{CH}_2\text{Cl}_2$ and solids were examined as KBr pellets and the values are reported in ν_{max} (cm^{-1}). HPLC analyses were done with Rainin System fitted with Dynamax[®] SD-200 pump and detected with Groton PDA solonet Diode Array Detector.

General Procedure for the Synthesis of methyl-L-amino ester hydrochloride

To an ice cold, stirring suspension of the L-amino acid (1 equivalent) in methanol (1 mL/mmol) was added thionylchloride (1.1 equivalents) drop wise from a dropping funnel. The clear solution that resulted was stirred at room temperature for 3 h. Methanol was removed in vacuo and the resulting solid was washed with dry ether. Drying under vacuo yielded the amino ester hydrochloride in nearly quantitative yields, which was used for further reaction in the next step without any further purification.

In general it was observed that coupling reactions with methyl-amino ester hydrochlorides were better yielding when these were freshly synthesised before use for subsequent synthesis, due to the hygroscopic nature of most of these salts.

General Procedure for the Synthesis of methyl-N-cinnamoyl-amino ester

Method A

To a stirring, ice cold solution of cinnamic acid (1 equivalent) and triethylamine (1 equivalent) in THF (1.5 mL/mmol) was added methylchloroformate (1 equivalent) and the mixture was stirred vigorously for 2 minutes.* After which, a solution of the amino ester hydrochloride (1.1 equivalents) in DMSO (0.5 mL/mmol) was added followed by triethylamine (2.2 equivalents) dissolved in THF (1 mL/mmol). The reaction vessel was allowed to warm to room temperature and vigorously stirred for further 3-4 h.

Triethylamine hydrochloride was filtered off on a sintered funnel under suction and washed with THF. Removal of solvent from the filtrate under vacuo yielded a residue, which was dissolved in EtOAc (~ 2mL/mmol) and washed with saturated aqueous solution of NaHCO₃, water and brine. Drying (Na₂SO₄) and evaporation of solvent under vacuo yielded the crude product which was further purified by column chromatography (EtOAc:Hexane) to yield the required product usually as good solids in good yields.

* On prolonging beyond this time, usually methyl cinnamate is formed in healthy quantities as a side product.

Method B

To a stirring ice cold solution of cinnamoyl chloride (1 equivalent) in dichloromethane (1 mL/mmol) was added the amino ester hydrochloride (1.1 equivalents) followed by a solution of triethylamine (2.2 equivalents) in dichloromethane (1 mL/mmol) drop wise through a dropping funnel. After complete addition of triethylamine, the reaction mixture was vigorously stirred for a further 5-6 h and then diluted with dichloromethane (1 mL/mmol). Work up as described in method A, with saturated aqueous solution of NaHCO₃, water and brine and purification by column chromatography yielded the N-cinnamoyl amino ester in good yields.

In general, it was observed that yields of methyl-N-cinnamoyl-amino esters were better by method A, than by method B.

General Procedure for the Synthesis of N-cinnamoyl-amino acid

Method A

To an ice cold solution of cinnamoylchloride (1 equivalent) in dichloromethane (1 mL/mmol) was added the L-amino acid (1 equivalent). To the stirring mixture a solution of triethylamine (2.2 equivalents) in dichloromethane (1 mL/mmol) was added drop wise at such a rate that the vessel temperature did not exceed 10 °C. After complete addition, the reaction mixture was warmed to room temperature and stirring was continued for 4-5 h. The solvent was evaporated in vacuo. To the resulting residue was added while stirring, aqueous solution of 1N HCl until no more acidification occurred (formation of milky white precipitate ceased). The aqueous suspension of yellowish white solid was filtered on a sintered funnel under suction and dried by spreading on cellulose filter paper for 10 h in a dessicator, to yield the N-cinnamoyl amino acid in excellent yields.

Method B

To a solution of the methyl-N-cinnamoyl amino ester (1 equivalent) in MeOH (4 mL/mmol) was added a solution of LiOH.H₂O (1.2-1.5 equivalents) in water (1 mL/mmol)* and stirred at room temperature until completion of reaction (TLC - complete disappearance of starting material). Methanol was removed under vacuo and the aqueous part was acidified with an aqueous solution of 1N HCl by drop wise addition and simultaneous vigorous stirring. After complete acidification (no more milky white precipitate occurs on addition of dilute HCl), the resulting residue - if it was a solid- was filtered off on a sintered funnel under suction and dried on cellulose filter paper for 10 - 12 h in a dessicator; or, if it was a gum, was extracted with dichloromethane, dried (anhydrous sodium sulphate) and concentrated under vacuo; to yield the N-cinnamoyl amino acid, usually in good yields as a solid or gum.

*It might be required to use warm water to dissolve LiOH.H₂O in case of larger scale reactions (> 20 mmol). In which case, after dissolving in warm water, it is better to cool the solution before addition to the methanolic solution of the methyl ester.

It is notable that the TLC patterns of most of these N-cinnamoyl peptides with free carboxyl group appear as white streaks, on visualising in iodine chamber.

General Procedure for the Polyaniline Supported Cobalt(II)salen (PASCOS) catalysed Epoxidation of N-Cinnamoyl-peptides:

To a solution of the N-cinnamoyl-peptide methyl ester (1 equivalent) in CH₃CN (5 mL/mmol) was added 2-methylpropanal (2 equivalents) and PASCOS catalyst[#] (~5 mg) and the contents were stirred under oxygen atmosphere at RT for 12 h. After this time, a fresh lot of the catalyst and 2-methylpropanal (2 equivalents)* were added to the reaction mixture and allowed to stir, until complete conversion of the olefin (TLC).[@] The catalyst was filtered off on a sintered funnel and acetonitrile was removed in vacuo. The resulting residue was taken in EtOAc and washed successively with saturated solution of NaHCO₃, water and brine. Separating the organic phase, drying and concentration in vacuo, yielded the corresponding oxirane containing peptide in high purity and yields (HPLC). These epoxides were further subjected to column chromatography (silica gel; EtOAc:Hexane) for purification to get to the pure epoxides in excellent yields.

[#] The catalyst should be washed thoroughly with acetonitrile until the wash is constantly colorless. Impurities in the catalyst were found to hinder the formation of epoxide.

* The aldehyde needed to be added in four equivalents for optimum and best conversions. Isobutyraldehyde can also be taken in lots of 3 equivalents and then 1 equivalent. However, lesser reaction times were realised by adding it in lots of 2 equivalents each.

[@] Pre-coated fluorescent silica plates and Silica gel-G coated glass plates were used as immobile phase. TLC plates were visualised in I2 chamber and under U.V. light, (254 nm). Characteristically, all the N-cinnamoyl peptides show up as excellent bright spots under U.V. light, whereas the epoxides show up as very light spots under U.V. light.

General Procedure for the Synthesis of β -phenylisoserine derived acyclic precursors for cyclisation

To a solution of the glycidic peptide (1 equivalent), (derived by the PASCOS catalysed epoxidation of corresponding N-cinnamoyl-peptide) in acetonitrile (5 mL/mmol) was added allyl-*p*-anisidine (1 equivalent) and CoCl₂ (anhydrous) (catalytic) and the contents were stirred until complete consumption of the epoxide (TLC - EtOAc:Hexane). The solvent was removed under vacuum and the resulting residue was taken in EtOAc and washed with water. The organic layer was separated, dried (Na₂SO₄) and concentrated to yield a residue, which was subjected to purification by column chromatography (silica gel-EtOAc:hexane) to yield the corresponding β -phenylisoserine derived acyclic precursor for cyclisation (via RCM) in good yields.

Synthesis of N-allyl-*p*-anisidine

To an ice-cold solution of *p*-anisidine (4.92 gm, 40 mmol) in acetone (60 mL) was added a solution of allylbromide (1.22 gm, 10 mmol) in acetone (20 mL) at the rate of 1 mL/min). The contents of the flask were allowed to stir at ambient conditions for further 12 h after complete addition of allylbromide. The solvent was removed under vacuo and a saturated solution of ZnCl₂ (50 mL) was added and the contents were stirred over night. A brown paste forms, which is filtered off on a sintered funnel to get a filtrate as a

mixture of brown organic and colorless aqueous layers. EtOAc was added to the filtrate and the organic phase was separated, dried (Na_2SO_4) and concentrated under vacuo to yield a brown liquid, which was subjected to column chromatography (Silicagel - hexane) to isolate allyl-*p*-anisidine as yellow oil in good yields (66 %). Small amounts (~5 %) of the di-allylated product were also formed.

^1H NMR, 60 MHz, CDCl_3 , δ 7.47 (d, $J = 8$ Hz, 2H), 7.29 (d, $J = 8$ Hz, 2H), 5.86 - 5.79 (m, 1H), 5.64 (d, $J = 12$ Hz, 1H), 5.60 (d, $J = 10$ Hz, 1H), 4.52 (bs, 1H), 4.32 (s, 2H)

Synthesis of methyl-L-prolinate hydrochloride

To an ice cold, stirring suspension of L-proline (4.6 gm, 40mmol) in methanol (40 mL) was added thionylchloride (3.2mL, 44 mmol) drop wise from a dropping funnel. The clear solution that resulted was stirred at room temperature for 3 h. Methanol was removed in vacuo and the resulting gummy liquid was washed with dry ether. Drying under vacuo yielded methyl-L-proline hydrochloride as a gum in nearly quantitative yields, which was used for further reaction in the next step without any further purification.

Synthesis of methyl-L-leucinate hydrochloride

To an ice cold, stirring suspension of L-leucine (3.93 gm, 30 mmol) in methanol (30 mL) was added thionylchloride (2.41 mL, 33 mmol) drop wise from a dropping funnel. The clear solution that resulted was stirred at room temperature for 3 h. Methanol was removed in vacuo and the resulting solid was washed with dry ether. Drying under vacuo yielded methyl-L-leucine hydrochloride in nearly quantitative yields as a crystalline white solid, which was used for further reaction in the next step without any further purification.

Synthesis of methyl-L-isoleucinate hydrochloride

To an ice cold, stirring suspension of L-isoleucine (2.62 gm, 20 mmol) in methanol (20 mL) was added thionylchloride (1.61 mL, 22 mmol) drop wise from a dropping funnel. The clear solution that resulted was stirred at room temperature for 3 h. Methanol was removed in vacuo and the resulting solid was washed with dry ether. Drying under vacuo yielded methyl-L-isoleucine hydrochloride in nearly quantitative yields as a crystalline white solid, which was used for further reaction in the next step without any further purification. It is advisable in the case of reactions with methyl-L-isoleucinate to freshly synthesize it every time before use.

Synthesis of methyl-L-phenylalaninate hydrochloride

To an ice cold, stirring suspension of L-phenylalanine (3.31 gm, 20 mmol) in methanol (20 mL) was added thionylchloride (1.61 mL, 22 mmol) drop wise from a dropping funnel. The clear solution that resulted was stirred at room temperature for 3 h. Methanol was removed in vacuo and the resulting solid was washed with dry ether. Drying under vacuo yielded methyl-L-phenylalaninate hydrochloride in nearly quantitative yields as a crystalline white solid, which was used for further reaction in the next step without any further purification.

Synthesis of N-Cinnamoyl-proline

To an ice cold solution of cinnamoylchloride (1.67 gm, 10 mmol) in dichloromethane (15 mL) was added L-proline (1.15 gm, 10 mmol). To the stirring mixture a solution of triethylamine (3.1 mL, 22 mmol) in dichloromethane (20 mL) was added drop wise at such a rate that the vessel temperature did not exceed 10 °C. After complete addition, the reaction mixture was warmed to room temperature and stirring was continued for 4-5 h. The solvent was evaporated in vacuo. To the resulting residue was added while stirring, aqueous solution of 1N HCl until no more acidification occurred (formation of milky white precipitate ceased). The aqueous suspension of yellowish white solid was filtered on a sintered funnel under suction and dried by spreading on cellulose filter paper for 10 h in a dessicator, to yield N-cinnamoyl proline as a white solid (M.P. = 162 °C) in excellent yields (96 %).

Synthesis of allyl-N-cinnamoyl-prolinate (3)

To a solution of N-cinnamoyl-L-proline (2.59 gm, 10 mmol) in acetone (15 mL) was added K₂CO₃ (1.52 gm, 11 mmol) and allylbromide (1.34 gm, 11 mmol) and the reaction mixture was set to reflux for 8 h during which time, the reaction was almost complete. The inorganic salts were filtered off on a sintered funnel under suction and solvent was removed under vacuo. The resulting residue was taken in EtOAc (25 mL) and washed with saturated aqueous solution of NaHCO₃ (2X10mL), water (2X10mL) and brine (1X10mL). Drying the organic layer (anhydrous Na₂SO₄) and concentration in vacuo yielded a thick residue which was purified by column chromatography (EtOAc in Hexane-25 %) (TLC - R_f = 0.4; hexane:ethylacetate 2.5:1) to yield allyl-N-cinnamoyl-prolinate as a gum, in good yields (72 %).

¹HNMR, 60 MHz, CDCl₃, δ 7.75 (d, J = 15 Hz, 1H), 7.50 (bs, 5H), 6.75 (d, J = 15 Hz, 1H), 6.00 (m, 1H), 5.45 (d, J = 15 Hz, 1H), 5.20 (d, J = 4 Hz, 1H), 4.65 (d, J = 7.5 Hz, 2H), 4.60 (m, 1H), 3.85 (m, 2H), 2.12 (m, 4H)

Synthesis of allyl-N-(3-phenylglycidyl)-prolinate (4)

To a solution of the allyl-N-cinnamoyl-prolinate (855 mg, 2 mmol) in CH₃CN (10 mL) was added 2-methylpropanal (288 mg, 4 mmol) and PASCOS catalyst (~5 mg) and the contents were stirred under oxygen atmosphere at RT for 12 h. After this time, a fresh lot of the catalyst and 2-methylpropanal (288 mg, 4 mmol) were added to the reaction mixture and allowed to stir, until complete conversion of the olefin (TLC - R_f = 0.5; EtOAc:Hexane - 1:2). The catalyst was filtered off on a cintered funnel and acetonitrile was removed in vacuo. The resulting residue was taken in EtOAc (25 mL) and washed successively with saturated solution of NaHCO₃ (2X10 mL), water (2X10 mL) and brine (1X10 mL). Separating the organic phase, drying (Na₂SO₄) and concentration in vacuo, yielded the corresponding oxirane containing peptide 4 in high purity and yields (HPLC). This was further subjected to column chromatography (silica gel; EtOAc:Hexane - 2:3) for purification to get to the pure epoxide 4 as a gum in excellent yields (85 %) [α]_D²⁵ = -64.28° (c = 0.007, CH₂Cl₂).

¹HNMR, 60 MHz, CDCl₃, δ 7.20 (bs, 5H), 5.90 (m, 1H), 5.30 (d, J = 16 Hz, 1H), 5.25 (d, J = 10 Hz, 1H), 4.60 (d, J = 6 Hz, 2H), 4.55 (dd, J = 3 Hz & 1.5 Hz, 1 H), 4.10 (d, J = 3 Hz, 1H), 3.70 (bs, 1H), 3.60 (m, 1H), 3.40 (d, J = 3 Hz, 1H), 2.00 (m, 4H).

Synthesis of allyl-N,N-(allyl,*p*-methoxyphenyl)- β -phenylisoserine-prolinate (5)

To a solution of allyl-N-(3-phenylglycidyl)-prolinate (452 mg, 1.5 mmol), in acetonitrile (7.5 mL) was added allyl-*p*-anisidine (244 mg, 1.5 mmol) and CoCl₂ (5 mg) and the contents were stirred until complete consumption of the epoxide (TLC - EtOAc:Hexane - 1:1.5). The solvent was removed under vacuum and the resulting residue was taken in EtOAc (20 mL) and washed with water (2X5 mL). The organic layer was separated, dried (Na₂SO₄) and concentrated in vacuo to yield a residue, which was subjected to purification by column chromatography (silica gel - EtOAc:hexane - 1:2) to yield allyl-N,N-(allyl,*p*-methoxyphenyl)- β -phenylisoserine-prolinate **5**, in good yields (52 %) as a gum $[\alpha]_D^{25} = -41.38^\circ$ ($c = 0.0145$, CH₂Cl₂).

¹H NMR, CDCl₃, 60 MHz, δ 7.25 (s, 1H), 7.1 (s, 2H), 6.65 (s, 2H), 5.65 (m, 2H), 5.3 (s, 1H), 5.2 (s, 1H), 5.05 (m, 1H), 4.9 (d, $J = 3$ Hz, 1H), 4.75 (d, $J = 6$ Hz, 1H), 4.65 - 4.40 (m, 4H), 4.25 (t, $J = 3.2$ Hz, 1H), 3.95 (d, $J = 5.6$ Hz, 1H), 3.71 (s, 3H), 3.3 (d, $J = 9$ Hz, 1H), 2.2 - 2.06 (m, 2H), 2.02 - 1.91 (m, 2H). MS m/z 464 (M⁺), 252, 182, 154

Synthesis of methyl-N-cinnamoyl-leucinate (6)

To a stirring, ice-cold solution of cinnamic acid (1.48 gm, 10mmol) and triethylamine (1.4 mL, 10mmol) in THF (15 mL) was added methylchloroformate (0.77 mL, 10 mmol) and the mixture was stirred vigorously for 2 minutes. After which, a solution of methyl leucinate hydrochloride (2 gm, 10 mmol) in DMSO (4-5 mL) was added followed by triethylamine (3.1 mL, 22 mmol) dissolved in THF (15 mL). The reaction vessel was allowed to warm to room temperature and vigorously stirred for further 3-4 h. Triethylamine hydrochloride was filtered off on a sintered funnel under suction and washed with THF. Removal of solvent from the filtrate under vacuo yielded a residue which was stirred with saturated aqueous solution of NaHCO₃ (20 mL) for 15 minutes. The bicarbonate layer was decanted and the resulting semi solid washed with water (2X10 mL). A yellow solid precipitated, which was filtered off on a suction funnel and dried in a dessicator under vacuum, on a cellulose filter paper for 10 h. Purification of the resulting solid by column chromatography (EtOAc:Hexane = 1:5.25) (TLC - R_f = 0.5; hexane:ethylacetate 5:1) yielded the above said compound as a crystalline white solid (M.P.=) in good yields (85%). $[\alpha]_D^{25} = +20^\circ$ ($c = 0.01$, CH₂Cl₂).

¹H NMR, 60 MHz, CDCl₃, δ 7.73 (d, $J = 8.2$ Hz, 1H), 7.60 (d, $J = 16$ Hz, 1H), 7.35 (s, 3H), 6.70 (d, $J = 16$ Hz, 1H), 4.90 (dd, $J = 10.2$ Hz & $J = 5.6$ Hz, 1H), 3.82 (s, 3H), 1.73 (dd, $J = 8$ Hz & $J = 1.6$ Hz, 2H), 1.21 - 1.18 (m, 1H), 0.96 (d, $J = 6.5$ Hz, 6H)

Synthesis of N-Cinnamoyl-leucine (7)

To a solution of the methyl-N-cinnamoyl leucinate (1.38 gm, 5 mmol) in MeOH (20 mL) was added a solution of LiOH.H₂O (205 mg, 5 mmol) in water (5 mL) and stirred at room temperature until completion of reaction (TLC - EtOAc:hexane - 1:3 - complete disappearance of starting material). Methanol was removed under vacuo and the aqueous part was acidified with an aqueous solution of 1N HCl by drop wise addition and simultaneous vigorous stirring. After complete acidification (no more milky white precipitate occurs on addition of dilute HCl), the resulting residue was extracted with dichloromethane (30mL), dried (anhydrous sodium sulphate) and concentrated under vacuo to yield N-cinnamoyl-L-leucine, in good yields (85 %) as a hygroscopic gum.

Synthesis of allyl-N-cinnamoyl-leucinate (8)

To a solution of N-cinnamoyl-L-leucine (313 mg, 1.2 mmol) in acetone (5 mL) was added K_2CO_3 (182 mg, 1.32 mmol) and allylbromide (161 mg, 1.32 mmol) and the reaction mixture was set to reflux for 8 h during which time, the reaction was almost complete. The inorganic salts were filtered off on a sintered funnel under suction and solvent was removed under vacuo. The resulting residue was taken in EtOAc (15 mL) and washed with saturated aqueous solution of $NaHCO_3$ (2X10mL), water (2X10mL) and brine (1X10mL). Drying the organic layer (anhydrous Na_2SO_4) and concentration in vacuo yielded a thick residue which was purified by column chromatography (EtOAc in Hexane-15 %) (TLC - R_f = 0.5; hexane : ethylacetate 2.5:1) to yield allyl-N-cinnamoyl-leucinate as a solid (M.P. 66-68 °C), in good yields (66 %) $[\alpha]_D^{25} = +15.38^\circ$ (c = 0.0065, CH_2Cl_2).

1H NMR, 60 MHz, $CDCl_3$, δ 7.3 (d, J = 16 Hz, 1H), 7.3 (s, 1H), 6.95 (s, 5H), 6.25 (d, J = 16 Hz, 1H), 5.5 (m, 1H), 5.05 (d, J = 9 Hz, 1H), 4.80 (t, J = 4.8 Hz, 1H), 4.61 (d, J = 7.5 Hz, 1H), 4.42 (d, J = 9 Hz, 2H), 0.90 (m, 1H), 0.78 (d, J = 12 Hz, 6H)

Synthesis of allyl-N-(3-phenylglycidyl)-leucinate (9)

To a solution of allyl-N-cinnamoyl-leucinate (602 mg, 2 mmol) in CH_3CN (10 mL) was added 2-methylpropanal (288 mg, 4 mmol) and PASCOS catalyst (~5 mg) and the contents were stirred under oxygen atmosphere at RT for 12 h. After this time, a fresh lot of the catalyst and 2-methylpropanal (288 mg, 4 mmol) were added to the reaction mixture and allowed to stir, until complete conversion of the olefin (TLC - R_f = 0.5; EtOAc:Hexane - 1:1). The catalyst was filtered off on a sintered funnel and acetonitrile was removed in vacuo. The resulting residue was taken in EtOAc (25 mL) and washed successively with saturated solution of $NaHCO_3$ (2X10 mL), water (2X10 mL) and brine (1X10 mL). Separating the organic phase, drying (Na_2SO_4) and concentration in vacuo, yielded the corresponding oxirane containing peptide 9 in high purity and yields (HPLC). This was further subjected to column chromatography (silica gel; EtOAc:Hexane - 1:4) for purification to get to the pure epoxide 9 as an oil in good yields (80 %) $[\alpha]_D^{25} = +8^\circ$ (c = 0.005, CH_2Cl_2).

1H NMR, $CDCl_3$, 60 MHz, δ 7.32 - 7.28 (m, 1H), 7.0 (s, 5H), 5.62 - 5.59 (m, 1H), 5.15 (s, 1H), 5.05 (d, J = 9 Hz, 1H), 4.92 (d, J = 9 Hz, 2H), 3.75 (d, J = 5 Hz, 1H), 3.43 (s, 1H), 1.5 (d, J = 7.5 Hz, 2H), 1.05 (m, 1H), 0.79 (d, J = 9 Hz, 6H)

Synthesis of allyl-N,N-(allyl,*p*-methoxyphenyl)- β -phenylisoserine-leucinate (10)

To a solution of allyl-N-(3-phenylglycidyl)-leucinate (476 mg, 1.5 mmol), in acetonitrile (7.5 mL) was added allyl-*p*-anisidine (244 mg, 1.5 mmol) and $CoCl_2$ (5 mg) and the contents were stirred until complete consumption of the epoxide (TLC - EtOAc:Hexane - 1:3). The solvent was removed under vacuum and the resulting residue was taken in EtOAc (20 mL) and washed with water (2X5 mL). The organic layer was separated, dried (Na_2SO_4) and concentrated in vacuo to yield a residue, which was subjected to purification by column chromatography (silica gel - EtOAc:hexane - 1:3) to yield allyl-N,N-(allyl,*p*-methoxyphenyl)- β -phenylisoserine-leucinate 10, in moderate yields (50 %) as a gum $[\alpha]_D^{25} = 0^\circ$ (c = 0.0155, CH_2Cl_2).

^1H NMR, 400 MHz, CDCl_3 , δ 7.46 - 7.41 (m, 1H), 7.31 - 7.28 (m, 2H), 7.24 (dd, $J = 5.1$ Hz & 2 Hz, 3H), 7.19 (d, $J = 8.8$ Hz, 1H), 7.09 (d, $J = 8$ Hz, 1H), 6.81 (d, $J = 8$ Hz, 2H), 5.85 (tt, $J = 13.6$ Hz & 5.6 Hz, 1H), 5.74 (tt, $J = 13.7$ Hz & 4 Hz, 1H), 5.28 (dd, $J = 9.5$ Hz & 1.4 Hz, 1H), 5.21 (dd, $J = 10.2$ Hz & 0.8 Hz, 1H), 4.99 (d, $J = 10$ Hz, 1H), 4.89 (d, $J = 4.7$ Hz, 1H), 4.76 (d, $J = 4.2$ Hz, 1H), 4.55 (dd, $J = 6$ Hz & 1.2 Hz, 2H), 4.46 (d, $J = 4.7$ Hz, 1H), 4.41 (dt, $J = 9$ Hz & 5.1 Hz, 1H), 3.77 (s, 3H), 3.61 (dd, $J = 13.4$ Hz & 4 Hz, 1H), 3.46 (dd, $J = 14.2$ Hz & 6 Hz, 1H), 1.36 (ddd, $J = 13.9$ Hz & 9 Hz & 5.1 Hz, 1H), 1.21 (ddd, $J = 13.8$ Hz, 6.6 Hz & 4.7 Hz, 1H), 0.99 - 0.85 (m, 1H), 0.74 (d, $J = 6.6$ Hz, 3H), 0.71 (d, $J = 6.6$ Hz, 3H); MS m/z 479, 252, 197, 170

Attempts to synthesize cyclic peptide (11)

To a clean dry flask flushed with N_2 , was added $(\text{Pcy})_3\text{Cl}_2\text{Ru}$ benzylidene catalyst (41 mg, 0.05 mmol) and CH_2Cl_2 (208 mL) and stirred for 15 min. at ambient conditions. A violet colored solution was formed. To this was added a solution of the diene **5** (116 mg, 0.25 mmol) in CH_2Cl_2 (208 mL), in a period of 1 h. through a syringe. The violet coloured solution turned orange. The contents of the flask were stirred after complete addition of the diene for 36 h. The reaction was quenched by stirring with 1 mL of water for 15 min. The organic phase was dried (Na_2SO_4), concentrated and subjected to flash column chromatography to isolate the starting material **5** as the major component of the mixture, along with $\text{P}(\text{Cy})_3$. No traces of the expected cyclised product were found.

To a clean dry flask flushed with N_2 , was added $(\text{Pcy})_3\text{Cl}_2\text{Ru}$ benzylidene catalyst (41 mg, 0.05 mmol) and CH_2Cl_2 (208 mL) and stirred for 15 min. at ambient conditions. A violet colored solution was formed. To this was added a solution of the diene **5** (116 mg, 0.25 mmol) in CH_2Cl_2 (208 mL); in a period of 1 h. through a syringe. The violet colored solution turned orange. The contents of the flask were stirred after complete addition of the diene **5** under reflux, in nitrogen atmosphere for 36 h. The reaction was quenched by stirring with 1 mL of water for 15 min. The organic phase was dried (Na_2SO_4), concentrated and subjected to flash column chromatography to isolate the starting material **5** as the major component of the mixture, along with $\text{P}(\text{Cy})_3$. No traces of the expected cyclised product were found.

Attempts to synthesize cyclic peptide (12)

To a clean dry flask flushed with N_2 , was added $(\text{Pcy})_3\text{Cl}_2\text{Ru}$ benzylidene catalyst (41 mg, 0.05 mmol) and CH_2Cl_2 (208 mL) and stirred for 15 min. at ambient conditions. A violet colored solution was formed. To this was added a solution of the diene **10** (120 mg, 0.25 mmol) in CH_2Cl_2 (208 mL), in a period of 1 h. through a syringe. The violet coloured solution turned orange. The contents of the flask were stirred after complete addition of the diene for 36 h. The reaction was quenched by stirring with 1 mL of water for 15 min. The organic phase was dried (Na_2SO_4), concentrated and subjected to flash column chromatography to isolate the starting material **10** as the major component of the mixture, along with $\text{P}(\text{Cy})_3$. No traces of the expected cyclised product were found.

To a clean dry flask flushed with N_2 , was added $(\text{Pcy})_3\text{Cl}_2\text{Ru}$ benzylidene catalyst (41 mg, 0.05 mmol) and CH_2Cl_2 (208 mL) and stirred for 15 min. at ambient conditions. A violet colored solution was formed. To this was added a solution of the diene **10** (120 mg, 0.25 mmol) in CH_2Cl_2 (208 mL), in a period of 1 h. through a syringe. The violet colored solution turned orange. The contents of the flask were stirred after complete addition of the diene **5** under reflux, in nitrogen atmosphere for 36 h. The reaction was quenched by stirring with 1 mL of water for 15 min. The organic phase was dried (Na_2SO_4), concentrated and subjected to flash column chromatography to isolate the starting

material **10** as the major component of the mixture, along with $P(Cy)_3$. No traces of the expected cyclised product were found.

Synthesis of methyl-N-(3'-hydroxy)cinnamoyl-prolinate (**13a**)

A stirring solution of (3'-hydroxy)cinnamic acid (820 mg, 5 mmol) and triethylamine (0.7 mL, 5 mmol) in THF (10 mL) was cooled to $-10\text{ }^{\circ}\text{C}$ in an ice-salt bath and to it was added methylchloroformate (0.38 mL, 10 mmol) and stirred vigorously for 50 - 60 seconds. Then a solution of methyl-L-prolinate hydrochloride (910 mg, 5.5 mmol) in DMSO (2.5 mL) was added followed by a solution of triethylamine (1.6 mL, 11 mmol) in THF (15 mL). The mixture was warmed to room temperature by removal of the ice bath and vigorously stirred for further 4 h. Triethylamine hydrochloride was filtered off on a sintered funnel under suction and washed twice with THF. Removal of solvent from the filtrate under vacuo yielded a residue, which was dissolved in EtOAc (20 mL) and washed with saturated aqueous solution of $NaHCO_3$ (2X10mL), water (2X10mL) and brine (1X10mL). Drying (Na_2SO_4) and evaporation of solvent under vacuo yielded the crude product which was further purified by column chromatography (EtOAc:Hexane-1:1.5) (TLC - R_f = 0.40; hexane:ethylacetate 1.5:1) to yield methyl-N-(3'-hydroxy)cinnamoyl-prolinate as a gum in moderate yields (52 %).

1H NMR, 60 MHz, $CDCl_3$ δ 7.62(d, J = 19 Hz, 1H), 7.16 (d, J = 8 Hz, 1H), 7.08 – 6.96 (m, 3H), 6.89 (d, J = 3 Hz, 1H), 6.69 (d, J = 19 Hz, 1H), 4.6 (dd, J = 6 Hz & 4.2 Hz, 1H), 3.85 (d, J = 9.6 Hz, 1H), 3.70 (s, 3H), 3.65 – 3.50 (m, 1H), 2.22 – 2.18 (m, 1H), 2.10 (dd, J = 9 Hz & 3 Hz, 2H), 2.00 – 1.92 (m, 1H)

Synthesis of N-(3'-hydroxy)cinnamoyl-proline (**14a**)

To a solution of the methyl-N-(3'-hydroxy)cinnamoyl-prolinate (247 mg, 1 mmol) in MeOH (4 mL) was added a solution of $LiOH.H_2O$ (41 mg, 1 mmol) in water (1 mL) and stirred at room temperature until completion of reaction (TLC - EtOAc:hexane - 1:1.5 - complete disappearance of starting material). Methanol was removed under vacuo and the aqueous part was acidified with an aqueous solution of 1N HCl by drop wise addition and simultaneous vigorous stirring. After complete acidification (no more milky white precipitate occurs on addition of dilute HCl), the resulting residue was extracted with dichloromethane (3 X 10mL), dried (anhydrous sodium sulphate) and concentrated under vacuo to yield the carboxylic acid, N-(3'-hydroxy)cinnamoyl-proline **14a**, in good yields (78 %) as a hygroscopic solid.

Synthesis of diallyl-N-(3'-allyloxy)cinnamoyl-prolinate (**15a**)

To a solution of N-(3'-hydroxy)cinnamoyl-proline (185 mg, 0.75 mmol) in acetone (5 mL) was added K_2CO_3 (228 mg, 1.66 mmol) and allylbromide (202 mg, 1.66 mmol) and the reaction mixture was set to reflux for 8 h during which time, the reaction was almost complete. The inorganic salts were filtered off on a sintered funnel under suction and solvent was removed under vacuo. The resulting residue was taken in EtOAc (10 mL) and washed with saturated aqueous solution of $NaHCO_3$ (2X5 mL), water (2X5 mL) and brine (1X5 mL). Drying the organic layer (anhydrous Na_2SO_4) and concentration in vacuo yielded a thick residue which was purified by column chromatography (EtOAc in Hexane-30 %) (TLC - R_f = 0.4; hexane:ethylacetate 2:1) to yield the diene diallyl-N-(3'-allyloxy)cinnamoyl-prolinate **15a** as a gum, in moderate yields (65 %).

^1H NMR, 80 MHz, CDCl_3 , δ 7.68 (d, J = 19.6 Hz, 1H), 7.28 – 6.98 (m, 4H), 6.69 (d, J = 19.6 Hz, 1H), 6.21 (dt, J = 10.2 Hz & 4.8 Hz, 1H), 5.89 (ddd, J = 15 Hz, 5.6 Hz & 2.4 Hz, 1H), 5.46 (d, J = 7 Hz, 1H), 5.42 (d, J = 6.0 Hz, 1H), 5.30 (dd, J = 16 Hz & 2 Hz, 1H), 5.21 (td, J = 17.5 Hz & 1.6 Hz, 1H), 4.76 – 4.62 (m, 1H), 4.66 (d, J = 8.8 Hz, 2H), 4.52 (dd, J = 5.6 Hz & 1.5 Hz, 2H), 3.82 (dt, J = 10 Hz & 3.2 Hz, 1H), 3.71 (dd, J = 6 Hz & 5.6 Hz, 1H), 2.25 (dd, J = 4.8 Hz & 1.2 Hz, 1H), 2.18 (d, J = 4.5 Hz, 1H), 2.12 – 1.96 (m, 2H)

Synthesis of methyl-N-(3'-hydroxy)cinnamoyl-leucinate (13b)

A stirring solution of (3'-hydroxy)cinnamic acid (820 mg, 5 mmol) and triethylamine (0.7 mL, 5 mmol) in THF (10 mL) was cooled to -10°C in an ice-salt bath and to it was added methylchloroformate (0.38 mL, 10 mmol) and stirred vigorously for 50 - 60 seconds. Then a solution of methyl-L-leucinate hydrochloride (1 gm, 5.5 mmol) in DMSO (2.5 mL) was added followed by a solution of triethylamine (1.6 mL, 11 mmol) in THF (15 mL). The mixture was warmed to room temperature by removal of the ice bath and vigorously stirred for further 4 h. Triethylamine hydrochloride was filtered off on a sintered funnel under suction and washed twice with THF. Removal of solvent from the filtrate under vacuo yielded a residue, which was dissolved in EtOAc (20 mL) and washed with saturated aqueous solution of NaHCO_3 (2X10mL), water (2X10mL) and brine (1X10mL). Drying (Na_2SO_4) and evaporation of solvent under vacuo yielded the crude product which was further purified by column chromatography (EtOAc:Hexane-1:2) (TLC: R_f =0.5; hexane:ethylacetate 1.5:1) to yield methyl-N-(3'-hydroxy)cinnamoyl-leucinate as a gum in moderate yields (52 %).

^1H NMR, 80 MHz, CDCl_3 , δ 7.46 (d, J = 16.4 Hz, 1H), 7.16 (d, J = 4.5 Hz, 1H), 6.96 – 6.81 (m, 3H), 6.70 (s, 1H), 6.28 (d, J = 16.4 Hz, 1H), 4.73 (dd, J = 8 Hz & 7.6 Hz, 1H), 3.65 (s, 3H), 1.66 (d, J = 11.5 Hz, 2H), 1.23 – 1.00 (m, 1H), 0.96 (d, J = 6.8 Hz, 6H)

Synthesis of N-(3'-hydroxy)cinnamoyl-leucine (14b)

To a solution of the methyl-N-(3'-hydroxy-cinnamoyl)-leucinate (782 mg, 2 mmol) in MeOH (12 mL) was added a solution of $\text{LiOH}\cdot\text{H}_2\text{O}$ (82 mg, 2 mmol) in water (3 mL) and stirred at room temperature until completion of reaction (TLC - EtOAc:hexane - 1:1.5 - complete disappearance of starting material). Methanol was removed under vacuo and the aqueous part was acidified with an aqueous solution of 1N HCl by drop wise addition and simultaneous vigorous stirring. After complete acidification (no more milky white precipitate occurs on addition of dilute HCl), the resulting residue was extracted with dichloromethane (3 X 10mL), dried (anhydrous sodium sulphate) and concentrated under vacuo to yield the carboxylic acid N-(3'-hydroxy-cinnamoyl)-leucine **14b**, in good yields (78 %) as a hygroscopic solid.

Synthesis of allyl-N-(3'-allyloxy)cinnamoyl-leucinate (15b)

To a solution of N-(3'-hydroxy-cinnamoyl)-leucine (416 mg, 1.5 mmol) in acetone (7.5 mL) was added K_2CO_3 (456 mg, 3.3 mmol) and allylbromide (402 mg, 3.3 mmol) and the reaction mixture was set to reflux for 8 h during which time, the reaction was almost complete. The inorganic salts were filtered off on a sintered funnel under suction and solvent was removed under vacuo. The resulting residue was taken in EtOAc (20mL) and washed with saturated aqueous solution of NaHCO_3 (2X5 mL), water (2X5 mL) and brine (1X5 mL). Drying the organic layer (anhydrous Na_2SO_4) and concentration in

vacuo yielded a thick residue which was purified by column chromatography (EtOAc in Hexane-35 %) (TLC - R_f = 0.4; hexane : ethylacetate 2.5:1) to yield the diene allyl-N-(3'-allyloxy)cinnamoyl-leucinate **15b** as a colourless gum, in moderate yields (65 %).

^1H NMR, 80 MHz, CDCl_3 δ 7.43 (d, J = 16.5 Hz, 1H), 6.97 (d, J = 4 Hz, 1H), 6.85 – 6.70 (m, 3H), 6.68 – 6.60 (s, 1H), 6.28 (d, J = 16.5 Hz, 1H), 6.10 – 5.85 (m, 1H), 5.80 – 5.48 (m, 1H), 5.45 (d, J = 5.6 Hz, 1H), 5.40 (d, J = 6 Hz, 1H), 5.28 (d, J = 10 Hz, 1H), 5.20 (d, J = 9.5 Hz, 1H), 4.68 (dd, J = 15 Hz & 8.5 Hz, 1H), 4.52 (d, J = 8 Hz, 2H), 4.45 (d, J = 5 Hz, 2H), 1.62 (dd, J = 7.5 Hz & 4 Hz, 2H), 1.20 – 1.10 (m, 1H), 0.98 (d, J = 6.5 Hz, 6H)

Synthesis of the Cyclic peptide (16)

To a clean dry flask flushed with N_2 , was added $(\text{Pcy})_3\text{Cl}_2\text{Ru}$ benzylidene catalyst (41 mg, 0.05 mmol) and CH_2Cl_2 (208 mL) and stirred for 15 min. at ambient conditions. A violet colored solution was formed. To this was added a solution of the diene **15a** (82 mg, 0.25 mmol) in CH_2Cl_2 (208 mL), in a period of 1 h. through a syringe. The violet coloured solution turned orange. The contents of the flask were stirred after complete addition of the diene for 36 h. The reaction was quenched by stirring with 1 mL of water for 15 min. The organic phase was dried (Na_2SO_4), concentrated and subjected to flash column chromatography to isolate the starting material **15a** as the major component of the mixture. No traces of the expected cyclised product were found.

To a clean dry flask flushed with N_2 , was added $(\text{Pcy})_3\text{Cl}_2\text{Ru}$ benzylidene catalyst (41 mg, 0.05 mmol) and CH_2Cl_2 (208 mL) and stirred for 15 min. at ambient conditions. A violet colored solution was formed. To this was added a solution of the diene **15a** (82 mg, 0.25 mmol) in CH_2Cl_2 (208 mL), in a period of 1 h. through a syringe. The violet colored solution turned orange. The contents of the flask were stirred after complete addition of the diene **15a** under reflux, in nitrogen atmosphere for 36 h. The reaction was quenched by stirring with 1 mL of water for 15 min. The organic phase was dried (Na_2SO_4), concentrated and subjected to flash column chromatography (EtOAc:hexane - 3:2) to isolate the required cyclised product **16** as a gum in very moderate yields (25 %), along with the starting material **15a** as the major component of the mixture.

^1H NMR, 80 MHz, CDCl_3 δ 7.47 (d, J = 18 Hz, 1H), 7.12 (d, J = 4 Hz, 1H), 7.04 – 6.92 (m, 3H), 6.83 (d, J = 3.2 Hz, 1H), 6.35 (d, J = 18 Hz, 1H), 6.26 (dd, J = 4.8 Hz & 2 Hz, 1H), 5.80 (dt, J = 8 Hz & 4.8 Hz, 1H), 4.77 (dd, J = 8 Hz & 6 Hz, 1H), 4.72 – 4.40 (m, 4H), 1.63 (dd, J = 10 Hz & 4.5 Hz, 2H), 1.18 – 1.10 (m, 1H), 0.96 (d, J = 8 Hz, 6H)

Synthesis of the Cyclic peptide (17)

To a clean dry flask flushed with N_2 , was added $(\text{Pcy})_3\text{Cl}_2\text{Ru}$ benzylidene catalyst (41 mg, 0.05 mmol) and CH_2Cl_2 (208 mL) and stirred for 15 min. at ambient conditions. A violet colored solution was formed. To this was added a solution of the diene **15b** (86 mg, .25 mmol) in CH_2Cl_2 (208 mL), in a period of 1 h. through a syringe. The violet coloured solution turned orange. The contents of the flask were stirred after complete addition of the diene for 36 h. The reaction was quenched by stirring with 1 mL of water for 15 min. The organic phase was dried (Na_2SO_4), concentrated and subjected to flash column chromatography to isolate the starting material **15b** as the major component of the mixture. No traces of the expected cyclised product were found.

To a clean dry flask flushed with N_2 , was added $(\text{Pcy})_3\text{Cl}_2\text{Ru}$ benzylidene catalyst (41 mg, 0.05 mmol) and CH_2Cl_2 (208 mL) and stirred for 15 min. at ambient conditions. A violet colored solution was formed. To this was added a solution of the diene **5** (86 mg,

.25 mmol) in CH_2Cl_2 (208 mL), in a period of 1 h. through a syringe. The violet colored solution turned orange. The contents of the flask were stirred after complete addition of the diene **15b** under reflux, in nitrogen atmosphere for 36 h. The reaction was quenched by stirring with 1 mL of water for 15 min. The organic phase was dried (Na_2SO_4), concentrated and subjected to flash column chromatography (EtOAc:hexane - 50 %) to isolate the required cyclised product in poor yields (10 %), along with the starting material **15b** as the major component of the mixture.

^1H NMR, 80 MHz (FT), CDCl_3 , δ 7.56 (d, $J = 18$ Hz, 1H), 7.05 – 6.78 (m, 4H), 6.51 (m, 4H), 6.51 (d, $J = 18$ Hz, 1H), 5.94 – 5.72 (m, 1H), 5.68 (ddd, $J = 18$ Hz, 8.5 Hz & 5 Hz, 1H), 5.16 (dd, $J = 7.2$ Hz & 6 Hz, 1H), 4.62 (d, $J = 7.5$ Hz, 2H), 4.46 (dd, $J = 6$ Hz & 1.2 Hz, 2H), 2.48 – 2.32 (m, 1H), 2.29 (d, $J = 4$ Hz, 1H), 2.16 – 1.82 (m, 2H)

Synthesis of methyl-N-cinnamoyl-proline-leucinate (**18a**)

A stirring solution of N-cinnamoyl-proline (2.45 gm, 10 mmol) and triethylamine (1.4 mL, 10 mmol) in THF (15 mL) was cooled to -10°C in an ice-salt bath and to it was added methylchloroformate (0.77 mL, 10 mmol) and stirred vigorously for 50 - 60 seconds. Then a solution of methyl-L-leucinate hydrochloride (2.00 gm, 11 mmol) in DMSO (0.5 mL) was added followed by a solution of triethylamine (3.1 mL, 22 mmol) in THF (15 mL). The mixture was warmed to room temperature by removal of the ice bath and vigorously stirred for further 4 h. Triethylamine hydrochloride was filtered off on a sintered funnel under suction and washed twice with THF. Removal of solvent from the filtrate under vacuo yielded a residue, which was dissolved in EtOAc (30 mL) and washed with saturated aqueous solution of NaHCO_3 (2X10mL), water (2X10mL) and brine (1X10mL). Drying (Na_2SO_4) and evaporation of solvent under vacuo yielded the crude product which was further purified by column chromatography (EtOAc:Hexane-1:1.5) (TLC - $R_f = 0.40$; hexane:ethylacetate 1.5:1) to yield methyl-N-cinnamoyl-L-proline-L-leucinate as a solid (M.P. = $78-80^\circ\text{C}$) in good yield (80 %); $[\alpha]_D^{25} = -170.5$ ($c = 0.01$, CH_2Cl_2).

^1H NMR, 400 MHz, CDCl_3 , δ 7.74 (d, $J = 15.3$ Hz, 1H), 7.66 (d, $J = 7.32$ Hz, 1H), 7.56 - 7.54 (m 2H), 7.39 - 7.35 (m, 3 H), 6.76 (d, $J = 15.6$ Hz, 1H), 4.57 - 4.47 (m, 2H), 3.73 (s, 3H), 3.70 - 3.61 (m, 2H), 2.51 - 2.46 (m, 1H), 2.06 - 2.03 (m, 1H), 2.21- 2.12 (m, 1H), 1.89 - 1.81 (m, 2H), 1.67 - 1.56 (m, 2H), 0.91 (d, $J = 5.6$ Hz, 3H), 0.88 (d, $J = 5.6$ Hz, 3H); IR (KBr) ν_{max} 3400, 3030, 2950, 2880, 1730, 1640, 1600, 1480, 1440 cm^{-1} ; FT IR (CH_2Cl_2): 3278, 3059, 2956.5, 2872.7, 1744.8, 1649.6, 1598.1, 1542.1, 1498.0, 1425.3

Synthesis of methyl-N-cinnamoyl-proline-phenylalaninate (**18b**)

A stirring solution of N-cinnamoyl-proline (2.45 gm, 10 mmol) and triethylamine (1.4 mL, 10 mmol) in THF (15 mL) was cooled to -5°C in an ice-salt bath and to it was added methylchloroformate (0.77 mL, 10 mmol) and stirred vigorously for 50 - 60 seconds. Then a solution of methyl-L-phenylalnininate hydrochloride (2.37 gm, 11 mmol) in DMSO (5 mL) was added followed by a solution of triethylamine (3.1 mL, 11 mmol) in THF (15 mL). The mixture was warmed to room temperature by removal of the ice bath and vigorously stirred for further 4 h. Triethylamine hydrochloride was filtered off on a sintered funnel under suction. Removal of solvent from the filtrate under vacuo yielded a residue, which was stirred with saturated aqueous solution of NaHCO_3 (20mL) for 15 minutes upon which, a solid precipitated. The aqueous bicarbonate layer was decanted and the solid was washed with water (2X10mL) and filtered on a sintered funnel under

suction. Drying of the solid on a cellulose filter paper in a desiccator under vacuo yielded the crude product which was further purified by column chromatography (EtOAc:Hexane-1:1.5) (TLC - R_f = 0.40; hexane:ethylacetate 1.5:1) to yield methyl-N-cinnamoyl-L-proline-L-phenylalaninate as a solid (M.P. = 41-43 °C) in good yields (80 %). $[\alpha]_D^{25} = -102$ ($c=0.004$, CH_2Cl_2).

^1H NMR, 400 MHz, CDCl_3 , δ 7.80 (d, J = 8 Hz, 1H), 7.73 (d, J = 15.4 Hz, 1H), 7.57 - 7.51 (m, 2H), 7.43 - 7.39 (m, 3H), 7.19 - 7.10 (m, 5H), 6.67 (d, J = 15.4 Hz, 1H), 4.84 (dt, J = 8.1 Hz & 5.4 Hz, 1H), 4.73 (d, J = 7.6 Hz, 1H), 3.73 (s, 3H), 3.54 - 3.51 (m, 2H), 3.19 (dd, J = 13.9 Hz & 5.4 Hz, 1H), 2.96 (dd, J = 13.9 Hz & 8 Hz, 1H), 2.42 - 2.39 (m, 1H), 1.98 - 1.900 (m, 2H), 1.79 - 1.72 (m, 1H); IR (KBr) ν_{max} 3400- 3300 (br), 3030, 2980, 1760, 1650, 1600 cm^{-1} .

Synthesis of N-cinnamoyl-proline-leucine (19a)

To a solution of the methyl-N-cinnamoyl-proline-leucinate (1.49 gm, 4 mmol) in MeOH (32 mL) was added a solution of $\text{LiOH}\cdot\text{H}_2\text{O}$ (164 mg, 4 mmol) in water (8 mL) and stirred at room temperature until completion of reaction (TLC - EtOAc : hexane - 1 : 1- complete disappearance of starting material). Methanol was removed under vacuo and the aqueous part was acidified with an aqueous solution of 1N HCl by drop wise addition and simultaneous vigorous stirring. After complete acidification (no more milky white precipitate occurs on addition of dilute HCl), the resulting residue was extracted with dichloromethane (3X10 mL), dried (anhydrous sodium sulphate) and concentrated under vacuo to yield N-cinnamoyl-L-leucine as a white solid (M.P. = 136-138 °C), in good yields (88 %).

Synthesis of N-cinnamoyl-proline-phenylalanine (19b)

To a solution of the methyl-N-cinnamoyl-proline-phenylalaninate (1.22 gm, 3 mmol) in MeOH (20 mL) was added a solution of $\text{LiOH}\cdot\text{H}_2\text{O}$ (123 mg, 3 mmol) in water (5 mL) and stirred at room temperature until completion of reaction (TLC - EtOAc : hexane - 1 : 1- complete disappearance of starting material). Methanol was removed under vacuo and the aqueous part was acidified with an aqueous solution of 1N HCl by drop wise addition and simultaneous vigorous stirring. After complete acidification (no more milky white precipitate occurred on addition of dilute HCl), the resulting residue was extracted with dichloromethane (X 10mL), dried (anhydrous sodium sulphate) and concentrated under vacuo to yield N-cinnamoyl-phenylalanine as a white solid, in good yields (85 %).

Synthesis of allyl-N-cinnamoyl-proline-leucinate (20a)

To a solution of N-cinnamoyl-proline-leucine 19a (1.25 gm, 3.5 mmol) in acetone (20 mL) was added K_2CO_3 (531 mg, 3.85 mmol) and allylbromide (470 mg, 3.85 mmol) and the reaction mixture was set to reflux for 8 h during which time, the reaction was almost complete. The inorganic salts were filtered off on a sintered funnel under suction and solvent was removed under vacuo. The resulting residue was taken in EtOAc (25 mL) and washed with saturated aqueous solution of NaHCO_3 (2X10 mL), water (2X10 mL) and brine (1X10 mL). Drying the organic layer (anhydrous Na_2SO_4) and concentration in vacuo yielded a thick residue which was purified by column chromatography (EtOAc in Hexane-40 %) (TLC - R_f = 0.5; hexane:ethylacetate 1:1) to yield allyl-N-cinnamoyl-proline-leucinate 20a as a gum, in moderate yields (63 %) $[\alpha]_D^{25} = -157.47^\circ$ ($c=0.0075$, CH_2Cl_2).

^1H NMR, 80 MHz, CDCl_3 , δ , 7.60 (d, $J = 15$ Hz, 1H), 7.30 (m, 6H), 6.65 (d, $J = 15$ Hz, 1H), 5.71 (m, 1H), 5.32 (d, $J = 9$ Hz, 1H), 5.1 (m, 1H), 4.91 (m, 1H), 4.75 (m, 1H), 4.55 (d, $J = 6$ Hz, 2H), 3.7 (t, $J = 6$ Hz, 2H), 2.0 (bs, 4H), 1.65 (dd, $J = 9$ Hz & 1.5 Hz, 2H), 1.25 (m, 1H), 0.95 (d, $J = 6$ Hz, 6H)

Synthesis of allyl-N-cinnamoyl-proline-phenylalaninate (20b)

To a solution of N-cinnamoyl-proline-phenylalanine **19b** (983 mg, 2.5 mmol) in acetone (20 mL) was added K_2CO_3 (380 mg, 2.75 mmol) and allylbromide (336 mg, 2.75 mmol) and the reaction mixture was set to reflux for 8 h during which time, the reaction was almost complete. The inorganic salts were filtered off on a sintered funnel under suction and solvent was removed under vacuo. The resulting residue was taken in EtOAc (25 mL) and washed with saturated aqueous solution of NaHCO_3 (2X10 mL), water (2X10 mL) and brine (1X10 mL). Drying the organic layer (anhydrous Na_2SO_4) and concentration in vacuo yielded a thick residue which was purified by column chromatography (EtOAc in Hexane-40 %) (TLC - $R_f = 0.45$; hexane:ethylacetate 1:1) to yield allyl-N-cinnamoyl-proline-leucinate **20b** as a solid (M.P. = 59 - 60 °C), in moderate yields (65 %) $[\alpha]_D^{25} = -86.67^\circ$ ($c = 0.003$, CH_2Cl_2).

^1H NMR, 400 MHz, CDCl_3 , δ , 7.85 (d, $J = 7.8$ Hz, 1H), 7.62 (d, $J = 15.6$ Hz, 1H), 7.44 (d, $J = 7.3$ Hz, 2H), 7.33 (d, $J = 6.4$ Hz, 1H), 7.25 (d, $J = 6.4$ Hz, 2H), 7.14 - 7.09 (m, 2H), 7.07 - 7.00 (m, 3H), 6.60 (d, $J = 15.6$ Hz, 1H), 5.73 (ddd, $J = 22$ Hz, 10.5 Hz & 5.8 Hz, 1H), 5.16 (d, $J = 17.1$ Hz, 1H), 5.08 (d, $J = 11.7$ Hz, 1H), 4.78 - 4.73 (m, 1H), 4.59 (d, $J = 7.8$ Hz, 1H), 4.60 (d, $J = 5.4$ Hz, 2H), 3.53 - 3.50 (m, 1H), 3.40 - 3.36 (m, 1H), 3.08 (dd, $J = 13.6$ Hz & 5.4 Hz, 1H), 2.90 (dd, $J = 13.6$ Hz & 7.8 Hz, 1H), 2.21 - 2.18 (m, 1H), 1.91 - 1.86 (m, 1H), 1.77 (bs, 1H), 1.69 - 1.60 (m, 1H)

Synthesis of allyl-N-(3-phenylglycidyl)-proline-leucinate (21a)

To a solution of allyl-N-cinnamoyl-proline-leucinate (796 mg, 2 mmol) in CH_3CN (10 mL) was added 2-methylpropanal (288 mg, 4 mmol) and PASCOS catalyst (~5 mg) and the contents were stirred under oxygen atmosphere at RT for 12 h. After this time, a fresh lot of the catalyst and 2-methylpropanal (288 mg, 4 mmol) were added to the reaction mixture and allowed to stir, until complete conversion of the olefin (TLC - $R_f = 0.55$; EtOAc:Hexane - 1:1). The catalyst was filtered off on a sintered funnel and acetonitrile was removed in vacuo. The resulting residue was taken in EtOAc (25 mL) and washed successively with saturated solution of NaHCO_3 (2X10 mL), water (2X10 mL) and brine (1X10 mL). Separating the organic phase, drying (Na_2SO_4) and concentration in vacuo, yielded the corresponding oxirane containing peptide **21a** in high purity and yields (HPLC). This was further subjected to column chromatography (silica gel; EtOAc:Hexane - 2:3) for purification to get to the pure epoxide **21a** as a gum in excellent yields (86 %) $[\alpha]_D^{25} = -57.5^\circ$ ($c = 0.02$, CH_2Cl_2).

^1H NMR, 80 MHz, CDCl_3 , δ , 7.30 (m, 6H), 5.71 (m, 1H), 5.30 (d, $J = 6$ Hz, 1H), 5.10 (m, 1H), 4.60 (d, $J = 6$ Hz, 2H), 4.01 (dd, $J = 3$ & 0.6 Hz, 2H), 3.59 (t, $J = 6$ Hz, 2H), 2.01 (bs, 4H), 1.65 (dd, $J = 9$ & 1.5 Hz, 2H), 1.25 (m, 1H), 0.95 (d, $J = 6$ Hz, 6H); MS m/z 415 (M^+), 357, 326, 295, 267, 209, 181, 149

Synthesis of allyl-N-(3-phenylglycidyl)-proline-phenylalaninate (21b)

To a solution of the allyl-N-cinnamoyl-proline-phenylalaninate (650 mg, 1.5 mmol) in CH_3CN (7.5 mL) was added 2-methylpropanal (216 mg, 3 mmol) and PASCOS catalyst

subjected to purification by column chromatography (silica gel - EtOAc:hexane - 5:4) to yield allyl-N,N-(allyl,*p*-methoxyphenyl)- β -phenylisoserine-proline-phenylalaninate **22b**, in moderate yields (55 %) as a gum $[\alpha]_D^{25} = -58^\circ$ ($c = 0.005$, CH₂Cl₂).

¹H NMR, 400 MHz, CDCl₃, δ , 7.30 (m, 1H), 7.22 (s, 5H), 6.81 (d, $J = 10.4$ Hz, 2H), 6.73 (d, $J = 16$ Hz, 1H), 5.25 (d, $J = 8$ Hz, 1H), 5.16 (d, $J = 18$ Hz, 1H), 5.10 (d, $J = 10$ Hz, 1H), 4.94 (d, $J = 3.4$ Hz, 1H), 4.86 (d, $J = 3.4$ Hz, 1H), 4.62 (m, 2H), 4.55 - 4.53 (m, 2H), 4.27 (d, $J = 1.7$ Hz, 1H), 3.82 (m, 1H), 3.69 (s, 3H), 3.60 (m, 1H), 3.50 (m, 1H), 2.19 (m, 1H), 2.05 - 2.02 (m, 3H), 1.65 (m, 1H), 1.54 (m, 1H), 0.98 (d, $J = 6.8$ Hz, 3H), 0.90 (d, $J = 3.5$ Hz, 2H); MS m/z 612 (M^+), 360, 330, 302, 252

Synthesis of Cyclic Peptide (23a)

To a clean dry flask flushed with N₂, was added (Pcy)₃Cl₂Ru benzylidene catalyst (41 mg, 0.05 mmol) and CH₂Cl₂ (208 mL) and stirred for 15 min. at ambient conditions. A violet colored solution was formed. To this was added a solution of the diene allyl-N,N-(allyl,*p*-methoxyphenyl)- β -phenylisoserine-proline-leucinate **22a** (144 mg, 0.25 mmol) in CH₂Cl₂ (208 mL), in a period of 1 h. through a syringe. The violet colored solution turned orange. The contents of the flask were stirred after complete addition of the diene **15b** under reflux, in nitrogen atmosphere for 36 h. The reaction was quenched by stirring with 1 mL of water for 15 min. The organic phase was dried (Na₂SO₄), concentrated and subjected to flash column chromatography (EtOAc:hexane - 70 %) to isolate the required cyclised peptide **23a** in good yields (46 %) $[\alpha]_D^{25} = -72.18^\circ$ ($c = 0.005$, CH₂Cl₂) as a gum, along with the starting material **22a** as the minor component of the mixture.

¹H NMR, 400 MHz, CDCl₃, δ 7.26 (s, 5H), 7.01 (d, $J = 8.8$ Hz, 1H), 6.76- 6.67 (m, 3H), 6.50 (d, $J = 8.8$ Hz, 1H), 6.24 (m, 1H), 5.90 (m, 1H), 5.37 - 5.20 (m, 1H), 5.11 (t, $J = 7.2$ Hz, 1H), 4.88 (dd, $J = 16$ Hz & 6.8 Hz, 1H), 4.61 (dd, $J = 11.2$ Hz & 6.8 Hz, 2H), 4.44 (dd, $J = 19.2$ Hz & 7.6 Hz, 1H), 4.20 (t, $J = 7.6$ Hz, 1H), 4.10 (dd, $J = 1.4$ Hz & 6.8 Hz, 1H), 3.75 (m, 1H), 3.71 (s, 3H), 3.39 (dt, $J = 26$ Hz & 9.6 Hz, 1H), 2.41 (m, 2H), 2.04 (m, 2H), 1.68 - 1.57 (m, 2H), 1.25 (m, 1H), 0.96 (d, $J = 5.2$ Hz, 3H), 0.91 (t, $J = 5.8$ Hz, 3H); MS m/z 549 (M^+), 492, 386, 325, 267, 211, 196, 165, 155, 131, 126

Synthesis of Cyclic peptide (23b)

To a clean dry flask flushed with N₂, was added (Pcy)₃Cl₂Ru benzylidene catalyst (41 mg, 0.05 mmol) and CH₂Cl₂ (208 mL) and stirred for 15 min. at ambient conditions. A violet colored solution was formed. To this was added a solution of the diene allyl-N,N-(allyl,*p*-methoxyphenyl)- β -phenylisoserine-proline-phenylalaninate **22b** (149 mg, 0.25 mmol) in CH₂Cl₂ (208 mL), in a period of 1 h. through a syringe. The violet colored solution turned orange. The contents of the flask were stirred after complete addition of the diene **22b** under reflux, in nitrogen atmosphere for 36 h. The reaction was quenched by stirring with 1 mL of water for 15 min. The organic phase was dried (Na₂SO₄), concentrated and subjected to flash column chromatography (EtOAc:hexane - 50 %) to isolate the required cyclised product in good yields (54 %) $[\alpha]_D^{25} = -57^\circ$ ($c = 0.005$, CH₂Cl₂) as a gum, along with the starting material **22b** as the minor component of the mixture.

¹H NMR, 400 MHz, CDCl₃, δ 7.40 (d, $J = 7.4$ Hz, 1H), 7.20-7.32 (m, 10H), 7.00 (d, $J = 8.8$ Hz, 2H), 6.84 (d, $J = 8.8$ Hz, 2H), 5.91 (m, 1H), 5.62 (m, 1H), 5.31 (bd, $J = 17.2$ Hz, 1H), 5.25 (d, $J = 10.7$ Hz, 1H), 5.02 (d, $J = 5.2$ Hz, 1H), 4.99 (bs, 1H), 4.75 (dd, J

12 and 4.8 Hz, 1H), 4.62 (m, 2H), 4.18 (dd, $J = 11.9$ and 6 Hz, 1H), 3.77 (s, 3H), 3.60 (dd, $J = 11.8$ and 5.2 Hz, 1H), 3.27 (dd, $J = 8.4$ and 6 Hz, 1H), 2.87 (d, $J = 7.8$ Hz, 1H), 1.65 (d, $J = 7.6$ Hz, 1H), 1.86 (m, 1H), 1.60-1.75 (m, 3H); MS m/z 584 (M^+), 527, 496, 66, 450, 406, 368, 342, 263, 212; IR (Neat) ν_{\max} 3300 (s), 3010, 2960, 2880, 1775, 680, 1600, 1560

Synthesis of methyl-N-cinnamoyl-phenylalaninate

To a stirring ice cold solution of cinnamoyl chloride (1.65 gm, 10 mmol) in dichloromethane (15 mL) was added methylphenylalaninate hydrochloride (2.37 gm, 11 mmol) followed by a solution of triethylamine (3.1 mL, 22 mmol) in dichloromethane (15 mL) drop wise through a dropping funnel. After complete addition of triethylamine, the reaction mixture was vigorously stirred for a further 5-6 h and then diluted with dichloromethane (15 mL). The organic layer was washed with saturated aqueous solution of NaHCO_3 , water and brine. Drying (Na_2SO_4) and evaporation of solvent under vacuo yielded the crude product which was further purified by column chromatography EtOAc:Hexane = 1:5 (TLC - $R_f = 0.5$; hexane ethylacetate 4:1) to yield the required product as a crystalline white solid (M.P. = 78°C) in good yields (80 %).

^1H NMR, 80 MHz, CDCl_3 , δ 7.68 (d, $J = 15.8$ Hz, 1H), 7.62 - 7.50 (m, 5H), 7.45 - 7.39 (m, 5H), 5.10 (dd, $J = 13.5$ Hz & 9 Hz, 1H), 3.69 (s, 3H), 3.15 (d, $J = 6$ Hz, 2H)

Synthesis of N-cinnamoyl-phenylalanine (7b)

To a stirring ice cold solution of triethylamine (3.1 mL, 22 mmol) in acetonitrile (30 mL) was added L-phenylalanine (1.65 gm, 10 mmol). A solution of cinnamoyl chloride (1.67 gm, 10 mmol) in acetonitrile (15 mL) was added drop wise to this solution at such a rate that the reaction vessel temperature did not exceed 10°C . After complete addition, the reaction mixture was warmed to room temperature and stirring was continued for 4-5 h. Following acidification and isolation as described in method A yielded N-cinnamoyl-L-phenylalanine as a sticky white solid in good yields (85 %).

Synthesis of methyl-N-cinnamoyl-leucine-prolinate (24a)

A stirring solution of N-cinnamoyl-L-leucine (1.30 gm, 5 mmol) and triethylamine (0.7 mL, 5 mmol) in THF (10 mL) was cooled to -10°C in an ice-salt bath and to it was added methylchloroformate (0.38 mL, 5 mmol) and stirred vigorously for 50 - 60 seconds. Then a solution of methyl-L-prolinate hydrochloride (916 mg, 5.5 mmol) in DMSO (2 mL) was added followed by a solution of triethylamine (1.6 mL, 11 mmol) in THF (10 mL). The mixture was warmed to room temperature by removal of the ice bath and vigorously stirred for further 4 h. Triethylamine hydrochloride was filtered off on a sintered funnel under suction. Removal of solvent from the filtrate under vacuo yielded a residue, which was dissolved in EtOAc (20mL) and washed with saturated aqueous solution of NaHCO_3 (2X10mL), water (2X10mL) and brine (1X10mL). Drying (Na_2SO_4) and evaporation of solvent under vacuo yielded the crude product which was further purified by column chromatography (EtOAc:Hexane-1:1.5) (TLC - $R_f = 0.50$; hexane:ethylacetate 1.5:1) to yield methyl-N-cinnamoyl-L-leucine-L-prolinate as a gum, in good yields (61 %).

^1H NMR, 80 MHz, CDCl_3 , δ 7.72(d, $J = 16\text{Hz}$, 1H), 7.59(d, $J = 8.9\text{Hz}$, 1H), 7.50-7.16 (m, 5H), 6.47 (d, $J = 16\text{Hz}$, 1H), 5.16 (dd, $J = 9\text{Hz}$ & $J = 5.6\text{Hz}$, 1H), 4.58 (dd, $J = 10.2\text{Hz}$ & $J = 4.8\text{Hz}$, 1H), 4.13 (d, $J = 11.2\text{Hz}$, 1H), 3.65 (s, 3H), 3.64 - 3.60 (m, 1H), 2.30 - 2.12 (m, 3H), 2.10 - 1.99 (m, 1H), 1.76 - 1.49 (m, 3H), 0.96 (d, $J = 5.6\text{Hz}$, 6H)

Synthesis of methyl-N-cinnamoyl-phenylalanine-prolinate (24b)

A stirring solution of N-cinnamoyl-L-phenylalanine (1.33 gm, 4.5 mmol) and triethylamine (0.63 mL, 4.5 mmol) in THF (10 mL) was cooled to -10 °C in an ice-salt bath and to it was added methylchloroformate (0.35 mL, 4.5 mmol) and stirred vigorously for 50 - 60 seconds. Then a solution of methyl-4-trans-hydroxy-L-prolinate hydrochloride (998 mg, 4.95 mmol) in DMSO (2.5 mL) was added followed by a solution of triethylamine (1.4 mL, 9.9 mmol) in THF (15 mL). The mixture was warmed to room temperature by removal of the ice bath and vigorously stirred for further 4 h. Triethylamine hydrochloride was filtered off on a sintered funnel under suction. Removal of solvent from the filtrate under vacuo yielded a residue, which was dissolved in EtOAc (25 mL) and washed with saturated aqueous solution of NaHCO₃ (2X10 mL), water (2X10 mL) and brine (1X10 mL). Drying (Na₂SO₄) and evaporation of solvent under vacuo yielded the crude product which was further purified by column chromatography (EtOAc:Hexane-1:1) (TLC - R_f = 0.45; hexane : ethylacetate 1:1) to yield methyl-N-cinnamoyl-phenylalanine-prolinate as a gum, in good yields (67 %).

¹HNMR, 80 MHz, CDCl₃, δ 7.62 (d, J = 6Hz, 1H), 7.46 (d, J = 16.5Hz, 1H), 7.50 - 7.35 (m, 5H), 7.33 (s, 5H), 7.18 (d, J = 8Hz, 1H), 6.46 (d, J = 16.5Hz, 1H), 5.15 (dd, J = 15Hz & J = 8Hz, 1H), 4.45 (dd, J = 13.5Hz & J = 6Hz, 1H), 4.00 (d, J = 8.8Hz, 1H), 3.68 (s, 3H), 3.57 (d, J = 8Hz, 1H), 3.12 (d, J = 8.5Hz, 2H), 2.25 - 2.13 (m, 1H), 2.18 (bs, 1H), 2.00 (bs, 1H), 1.96 (m, 1H)

Synthesis of N-cinnamoyl-leucine-proline (25a)

To a solution of the methyl-N-cinnamoyl-leucine-prolinate (1.12 gm, 3 mmol) in MeOH (20 mL) was added a solution of LiOH.H₂O (123 mg, 3 mmol) in water (5 mL) and stirred at room temperature until completion of reaction (TLC - EtOAc : hexane - 1 : 1- complete disappearance of starting material). Methanol was removed under vacuo and the aqueous part was acidified with an aqueous solution of 1N HCl by drop wise addition and simultaneous vigorous stirring. After complete acidification (no more milky white precipitate occurs on addition of dilute HCl), the resulting residue was extracted with dichloromethane (3X10 mL), dried (anhydrous sodium sulphate) and concentrated under vacuo to yield N-cinnamoyl-leucine-proline, in good yields (78 %) as a sticky hygroscopic solid.

Synthesis of N-cinnamoyl-phenylalanine-proline (25b)

To a solution of the methyl-N-cinnamoyl-phenylalanine-prolinate (1.22 gm, 3 mmol) in MeOH (20 mL) was added a solution of LiOH.H₂O (123 mg, 3 mmol) in water (5 mL) and stirred at room temperature until completion of reaction (TLC - EtOAc : hexane - 1 : 1- complete disappearance of starting material). Methanol was removed under vacuo and the aqueous part was acidified with an aqueous solution of 1N HCl by drop wise addition and simultaneous vigorous stirring. After complete acidification (no more milky white precipitate occurs on addition of dilute HCl), the resulting residue was extracted with dichloromethane (3X10 mL), dried (anhydrous sodium sulphate) and concentrated under vacuo to yield N-cinnamoyl-phenylalanine-proline, in good yields (72 %) as a gum.

Synthesis of allyl-N-cinnamoyl-leucine-prolinate (26a)

To a solution of N-cinnamoyl-leucine-proline (716 mg, 2 mmol) in acetone (10 mL) was added K₂CO₃ (304 mg, 2.2 mmol) and allylbromide (268 mg, 2.2 mmol) and the reaction

mixture was set to reflux for 8 h during which time, the reaction was almost complete. The inorganic salts were filtered off on a sintered funnel under suction and solvent was removed under vacuo. The resulting residue was taken in EtOAc (20 mL) and washed with saturated aqueous solution of NaHCO_3 (2X10 mL), water (2X10 mL) and brine (1X10 mL). Drying the organic layer (anhydrous Na_2SO_4) and concentration in vacuo yielded a thick residue which was purified by column chromatography (EtOAc in Hexane - 45 %) (TLC - R_f = 0.4; hexane:ethylacetate 1:1) to yield allyl-N-cinnamoyl-leucine-prolinate as a gum, in moderate yields (61 %).

^1H NMR, 400 MHz, CDCl_3 , δ 7.73 (d, J = 15.6 Hz, 1H), 7.53 - 7.52 (m, 1H), 7.46 - 7.45 (m, 1H), 7.38 - 7.32 (m, 3H), 7.12 (bs, 1H), 7.00 (m, 1H), 6.76 (d, J = 15.6 Hz, 1H), 5.82 (ddd, J = 22.4 Hz, 11.2 Hz & 5.2 Hz, 1H), 5.17 (d, J = 17.2 Hz, 1H), 5.10 (d, J = 10 Hz, 1H), 5.01 - 4.97 (m, 1H), 4.74 (d, J = 8.2 Hz, 1H), 3.91 - 3.89 (m, 1H), 3.83 - 3.80 (m, 2H), 3.64 (d, J = 8 Hz, 1H), 2.04 - 2.00 (m, 1H), 1.91 - 1.89 (m, 1H), 1.64 - 1.62 (m, 1H), 1.58 - 1.55 (m, 1H), 1.28 - 1.20 (m, 2H), 1.13 - 1.07 (m, 1H), 0.99 (d, J = 6.4 Hz, 3H), 0.94 (d, J = 6.4 Hz, 3H)

Synthesis of allyl-N-cinnamoyl-phenylalanine-prolinate (26b)

To a solution of N-cinnamoyl-phenylalanine-proline (786 mg, 2 mmol) in acetone (10 mL) was added K_2CO_3 (304 mg, 2.2 mmol) and allylbromide (268 mg, 2.2 mmol) and the reaction mixture was set to reflux for 8 h during which time, the reaction was almost complete. The inorganic salts were filtered off on a sintered funnel under suction and solvent was removed under vacuo. The resulting residue was taken in EtOAc (20 mL) and washed with saturated aqueous solution of NaHCO_3 (2X10 mL), water (2X10 mL) and brine (1X10 mL). Drying the organic layer (anhydrous Na_2SO_4) and concentration in vacuo yielded a thick residue which was purified by column chromatography (EtOAc in Hexane-45 %) (TLC - R_f = 0.5; hexane : ethylacetate 2:3) to yield allyl-N-cinnamoyl-phenylalanine-prolinate as a gum, in moderate yields (64 %).

^1H NMR, 400 MHz, CDCl_3 , δ 7.64 (d, J = 8.3 Hz, 1H), 7.56 (d, J = 15.7 Hz, 1H), 7.45 - 7.43 (m, 2H), 7.33 - 7.29 (m, 4H), 7.27 - 7.18 (m, 4H), 6.46 (d, J = 15.7 Hz, 1H), 5.81 (dd, J = 11.2 Hz & 5.6 Hz, 1H), 5.26 (d, J = 11.7 Hz, 1H), 5.17 (d, J = 5.2 Hz, 1H), 5.14 (d, J = 4.4 Hz, 1H), 4.56 (d, J = 5.36 Hz, 1H), 4.54 - 4.51 (m, 1H), 4.36 (dd, J = 7.6 Hz & 4.4 Hz, 1H), 3.74 - 3.72 (m, 1H), 3.68 (d, J = 6.8 Hz, 1H), 3.18 (dd, J = 14.16 Hz & 7.1 Hz, 1H), 3.08 (dd, J = 12.9 Hz & 9.3 Hz, 1H), 1.94 - 1.86 (m, 3H), 1.56 - 1.55 (m, 1H).

Synthesis of allyl-N-(3-phenylglycidyl)-leucine-prolinate (27a)

To a solution of the allyl-N-cinnamoyl-leucine-prolinate (597 mg, 1.5 mmol) in CH_3CN (7.5 mL) was added 2-methylpropanal (216 mg, 3 mmol) and PASCOS catalyst (~5 mg) and the contents were stirred under oxygen atmosphere at RT for 12 h. After this time, a fresh lot of the catalyst and 2-methylpropanal (216 mg, 3 mmol) were added to the reaction mixture and allowed to stir, until complete conversion of the olefin (TLC - R_f = 0.55; EtOAc:Hexane - 2:3). The catalyst was filtered off on a sintered funnel and acetonitrile was removed in vacuo. The resulting residue was taken in EtOAc (25 mL) and washed successively with saturated solution of NaHCO_3 (2X10 mL), water (2X10 mL) and brine (1X10 mL). Separating the organic phase, drying (Na_2SO_4) and concentration in vacuo, yielded the corresponding oxirane containing peptide **27a** in high purity and yields (HPLC). This was further subjected to column chromatography (silica

gel; EtOAc:Hexane - 2:3) for purification to get to the pure epoxide **27a** as a gum in good yields (70 %) [α]_D²⁵ = -120° (c = 0.01, CH₂Cl₂).

¹H NMR, 400 MHz, CDCl₃, δ 7.54 (d, J = 7.3 Hz, 1H), 7.48 (dd, J = 8 Hz & 3 Hz, 1H), 7.35 - 7.32 (m, 3H), 7.28 - 7.26 (m, 2H), 5.80 (d, J = 11.5 Hz & 5.6 Hz, 1H), 5.17 (dd, J = 17.1 Hz & 1.5 Hz, 1H), 5.11 (dd, J = 12.1 Hz & 1.5 Hz, 1H), 4.63 (dt, J = 8.8 Hz & 2.9 Hz, 1H), 4.57 (dd, J = 8 Hz & 5 Hz, 1H), 3.89 (d, J = 1.7 Hz, 1H), 3.85 (bs, 2H), 3.73 - 3.66 (m, 1H), 3.50 (d, J = 1.7 Hz, 1H), 3.49 - 3.45 (m, 1H), 2.31 - 2.28 (m, 1H), 2.17 (m, 1H), 2.04 (m, 1H), 1.92 - 1.89 (m, 1H), 1.68 (dt, J = 13.6 Hz & 4.1 Hz, 1H), 1.57 (dt, J = 13.6 Hz & 3.1 Hz, 1H), 1.13 - 1.10 (m, 1H), 1.00 (d, J = 6.4 Hz, 3H), 0.96 (d, J = 6.4 Hz, 3H).

Synthesis of allyl-N-(3-phenylglycidyl)-phenylalanine-prolinate (**27b**)

To a solution of the methyl-N-cinnamoyl-leucine-prolinate (650 mg, 1.5 mmol) in CH₃CN (7.5 mL) was added 2-methylpropanal (216 mg, 3 mmol) and PASCOS catalyst (~5 mg) and the contents were stirred under oxygen atmosphere at RT for 12 h. After this time, a fresh lot of the catalyst and 2-methylpropanal (216 mg, 3 mmol) were added to the reaction mixture and allowed to stir, until complete conversion of the olefin (TLC - R_f = 0.5; EtOAc:Hexane - 2:3). The catalyst was filtered off on a cindered funnel and acetonitrile was removed in vacuo. The resulting residue was taken in EtOAc (25 mL) and washed successively with saturated solution of NaHCO₃ (2X10 mL), water (2X10 mL) and brine (1X10 mL). Separating the organic phase, drying (Na₂SO₄) and concentration in vacuo, yielded the corresponding oxirane containing peptide **27b** in high purity and yields (HPLC). This was further subjected to column chromatography (silica gel; EtOAc:Hexane - 2:3) for purification to get to the pure epoxide **27b** in good yields (62 %) [α]_D²⁵ = -24° (c = 0.01, CH₂Cl₂).

¹H NMR, 400 MHz, CDCl₃, δ 7.41 (m, 1H), 7.32 - 7.17 (m, 10H), 5.92 - 5.84 (m, 1H), 5.30 (d, J = 17.1 Hz, 1H), 5.24 (d, J = 9.3 Hz, 1H), 5.03 (dd, J = 5.9 Hz & 1.5 Hz, 1H), 4.61 (dd, J = 18.8 Hz & 5.2 Hz, 2H), 4.35 (dt, J = 8.3 Hz & 4.2 Hz, 1H), 3.95 & 3.67 (d, J = 1.9 Hz, 1H), 3.65 - 3.52 (m, 2H), 3.52 & 3.44 (d, J = 1.9 Hz, 1H), 3.05 (d, J = 7.64 Hz, 2H), 1.93 - 1.85 (m, 4H)

Synthesis of allyl-N,N-(allyl,*p*-methoxyphenyl)- β -phenylisoserine-leucine-prolinate (**28a**)

To a solution of allyl-N-(3-phenylglycidyl)-leucine-prolinate (449 mg, 1 mmol), in acetonitrile (5 mL) was added allyl-*p*-anisidine (163 mg, 1 mmol) and CoCl₂ (5 mg) and the contents were stirred until complete consumption of the epoxide (TLC - EtOAc:Hexane - 1:1). The solvent was removed under vacuum and the resulting residue was taken in EtOAc (20 mL) and washed with water (2X5 mL). The organic layer was separated, dried (Na₂SO₄) and concentrated in vacuo to yield a residue, which was subjected to purification by column chromatography (silica gel - EtOAc:hexane - 5:4) to yield allyl-N,N-(allyl,*p*-methoxyphenyl)- β -phenylisoserine-leucine-prolinate **28a**, in moderate yields (55 %) as a gum [α]_D²⁵ = -8° (c = 0.0055, CH₂Cl₂).

¹H NMR, 400 MHz, CDCl₃, δ 7.99 (d, J = 7.3 Hz, 1H), 7.43 - 7.21 (m, 3H), 7.21 (d, J = 10 Hz, 2H), 7.17 (d, J = 10 Hz, 2H), 6.98 - 6.90 (m, 1H), 6.72 - 6.69 (m, 1H), 5.81 (dt, J = 11.2 Hz & 7.2 Hz, 1H), 5.87 - 5.77 (m, 2H), 5.23 (dd, J = 13.2 Hz & 1.6 Hz, 2H), 5.15 (dd, J = 10.5 Hz & 1.2 Hz, 2H), 4.86 (t, J = 5.6 Hz, 1H), 4.80 - 4.74 (m, 0.5H), 4.53 (dd, J

= 5.6 Hz & 1.2 Hz, 2H), 4.48 (t, J = 4.1 Hz, 2H), 4.45 - 4.32 (m, 1H), 3.71 (dd, J = 10.2 Hz & 4.9 Hz, 1H), 3.69 (s, 3H), 3.44 - 3.41 (m, 1H), 2.56 - 2.48 (m, 1H), 2.17 - 2.06 (m, 1H), 2.01 - 1.89 (m, 2H), 1.54 - 1.41 (m, 2H), 1.26 - 1.21 (m, 1H), 0.97 (d, J = 5.6 Hz, 3H), 0.88 (d, J = 5.6 Hz, 3H); MS m/z 577 (M^+), 252, 325, 295, 267; IR ν_{\max} 3360(br), 2920, 1775, 1660, 1600, 1480 cm^{-1}

Synthesis of allyl-N,N-(allyl,*p*-methoxyphenyl)- β -phenylisoserine-phenyl alanine-prolinate (28b)

To a solution of allyl-N-(3-phenylglycidyl)-phenylalanine-prolinate (449 mg, 1 mmol), in acetonitrile (5 mL) was added allyl-*p*-anisidine (163 mg, 1 mmol) and CoCl_2 (5 mg) and the contents were stirred until complete consumption of the epoxide (TLC - EtOAc:Hexane - 1:1). The solvent was removed under vacuum and the resulting residue was taken in EtOAc (20 mL) and washed with water (2X5 mL). The organic layer was separated, dried (Na_2SO_4) and concentrated in vacuo to yield a residue, which was subjected to purification by column chromatography (silica gel - EtOAc:hexane - 5:4) to yield allyl-N,N-(allyl,*p*-methoxyphenyl)- β -phenylisoserine-proline-leucinate **28b**, in moderate yields (48 %) as a gum $[\alpha]_D^{25} = -28.93^\circ$ (c = 0.0075, CH_2Cl_2).

^1H NMR, 400 MHz, CDCl_3 , δ 7.57 (d, J = 8.8 Hz, 1H), 7.38 (d, J = 5.8 Hz, 1H), 7.26 - 7.19 (m, 8H), 7.05 (d, J = 8.8 Hz, 2H), 6.94 - 6.91 (m, 1H), 6.80 (d, J = 4.4 Hz, 1H), 6.78 (d, J = 4.4 Hz, 1H), 5.90 - 5.74 (m, 2H), 5.30 (d, J = 16.8 Hz, 1H), 5.24 (d, J = 15.8 Hz, 1H), 5.15 (d, J = 10.4 Hz, 1H), 5.0 (d, J = 11.5 Hz, 1H), 4.92 (d, J = 16 Hz, 1H), 4.86 (d, J = 4.4 Hz, 1H), 4.78 (m, 1H), 4.86 (d, J = 5.6 Hz, 2H), 4.50 (dd, J = 14.6 Hz & 3.4 Hz, 2H), 4.59 (d, J = 5.6 Hz, 2H), 4.50 (dd, J = 14.6 Hz & 3.4 Hz, 2H), 4.22 (dd, J = 7.8 Hz & 4.5 Hz, 1H), 3.72 (s, 3H), 3.56 (m, 1H), 3.47 (m, 1H), 3.08 - 2.92 (m, 1H), 2.56 - 2.50 (m, 1H), 1.93 - 1.70 (m, 4H); MS m/z 612(M^+), 252, 359, 329, 301, 182, 211; IR ν_{\max} 3400(br), 3330, 3030, 2900, 2830, 1715, 1660, 1430 cm^{-1}

Attempts for the Synthesis of Cyclic peptide (29a)

To a clean dry flask flushed with N_2 , was added $(\text{Pcy})_3\text{Cl}_2\text{Ru}$ benzylidene catalyst (41 mg, 0.05 mmol) and CH_2Cl_2 (208 mL) and stirred for 15 min. at ambient conditions. A violet colored solution was formed. To this was added a solution of the diene **28a** (144 mg, 0.25 mmol) in CH_2Cl_2 (208 mL), in a period of 1 h. through a syringe. The violet colored solution turned orange. The contents of the flask were stirred after complete addition of the diene **28a** under reflux, in nitrogen atmosphere for 40 h. The reaction was quenched by stirring with 1 mL of water for 15 min. The organic phase was dried (Na_2SO_4), concentrated and subjected to flash column chromatography to isolate the starting material **28a** as the major component of the mixture along with minor amounts of highly polar products (insoluble in EtOAc/ CH_2Cl_2), along with $\text{P}(\text{Cy})_3$. No traces of the expected cyclised product were found.

Attempts for the Synthesis of Cyclic Peptide (29b)

To a clean dry flask flushed with N_2 , was added $(\text{Pcy})_3\text{Cl}_2\text{Ru}$ benzylidene catalyst (41 mg, 0.05 mmol) and CH_2Cl_2 (208 mL) and stirred for 15 min. at ambient conditions. A violet colored solution was formed. To this was added a solution of the diene **28b** (153 mg, 0.25 mmol) in CH_2Cl_2 (208 mL), in a period of 1 h. through a syringe. The violet colored solution turned orange. The contents of the flask were stirred after complete addition of the diene **28b** under reflux, in nitrogen atmosphere for 48 h. The reaction was quenched by stirring with 1 mL of water for 15 min. The organic phase was dried

(Na₂SO₄), concentrated and subjected to flash column chromatography to isolate the starting material **5** as the major component of the mixture along with minor amounts of highly polar products (insoluble in EtOAc/CH₂Cl₂, soluble in MeOH), along with P(Cy)₃. No traces of the expected cyclised product were found.

Synthesis of methyl-N-cinnamoyl-proline-leucine-isoleucinate (**30**)

A stirring solution of N-cinnamoyl-proline-leucine (1.79 gm, 5 mmol) and triethylamine (0.7 mL, 5 mmol) in THF (10 mL) was cooled to -10 °C in an ice-salt bath and to it was added methylchloroformate (0.38 mL, 5 mmol) and stirred vigorously for 50 - 60 seconds. Then a solution of methyl-L-isoleucinate hydrochloride (1.00 gm, 5.5 mmol) in DMSO (2.5 mL) was added followed by a solution of triethylamine (1.6 mL, 11 mmol) in THF (10 mL). The mixture was warmed to room temperature by removal of the ice bath and vigorously stirred for further 4 h. Triethylamine hydrochloride was filtered off on a sintered funnel under suction and washed twice with THF. Removal of solvent from the filtrate under vacuo yielded a residue, which was dissolved in EtOAc (30 mL) and washed with saturated aqueous solution of NaHCO₃ (2X10 mL), water (2X10 mL) and brine (1X10 mL). Drying (Na₂SO₄) and evaporation of solvent under vacuo yielded the crude product which was further purified by column chromatography (EtOAc:Hexane-3:2) (TLC - R_f = 0.5; hexane:ethylacetate 2:3) to yield methyl-N-cinnamoyl-proline-leucine-isoleucinate as a waxy solid in good yields (71 %).

¹H NMR, 400 MHz, CDCl₃, δ 7.74 (d, J = 16.2 Hz, 1H), 7.55 - 7.52(m, 2H), 7.39 - 7.35 (m, 3H), 6.68 (d, J = 6.8 Hz, 1H), 6.75 (d, J = 16.2 Hz, 1H), 4.74 (d, J = 6.1 Hz, 1H), 4.57 (dd, J = 8.8 Hz & 4.9 Hz, 1H), 4.36 (t, J = 7.6 Hz, 1H), 3.78 (dd, J = 14.9 Hz & 5.6 Hz, 1H), 3.69 (s, 3H), 3.66 (dd, J = 7.3 Hz & 5.9 Hz, 1H), 2.45 - 2.41 (m, 1H), 2.17 (s, 1H), 2.07 - 2.04 (m, 1H), 1.97 - 1.86 (m, 2H), 1.77 - 1.73 (m, 1H), 1.62 - 1.53 (m, 2H), 1.48 - 1.42 (m, 1H), 1.27 - 1.17 (m, 2H), 0.96 - 0.80 (m, 12H)

Synthesis of N-cinnamoyl-proline-leucine-isoleucine (**31**)

To a solution of the methyl-N-cinnamoyl-proline-leucine-isoleucinate (1.70 gm, 3.5 mmol) in MeOH (16 mL) was added a solution of LiOH.H₂O (143 mg, 3.5 mmol) in water (4 mL) and stirred at room temperature until completion of reaction (TLC - EtOAc:hexane - 3:1 - complete disappearance of starting material). Methanol was removed under vacuo and the aqueous part was acidified with an aqueous solution of 1N HCl by drop wise addition and simultaneous vigorous stirring. After complete acidification (no more milky white precipitate occurred on addition of dilute HCl), the resulting residue was extracted with dichloromethane (3X10 mL), dried (anhydrous sodium sulphate) and concentrated under vacuo to yield N-cinnamoyl-proline-leucine-isoleucine, in good yields (72 %) as a gum.

Synthesis of allyl-N-cinnamoyl-proline-leucine-isoleucinate (**32**)

To a solution of N-cinnamoyl-proline-leucine-isoleucine (1.41 gm, 3 mmol) in acetone (15 mL) was added K₂CO₃ (455 mg, 3.3 mmol) and allylbromide (403 mg, 3.3 mmol) and the reaction mixture was set to reflux for 8 h during which time, the reaction was almost complete. The inorganic salts were filtered off on a sintered funnel under suction and solvent was removed under vacuo. The resulting residue was taken in EtOAc (30 mL) and washed with saturated aqueous solution of NaHCO₃ (2X10 mL), water (2X10 mL) and brine (1X10 mL). Drying the organic layer (anhydrous Na₂SO₄) and concentration in vacuo yielded a thick residue which was purified by column

chromatography (EtOAc in Hexane-50 %) (TLC - R_f = 0.4; hexane : ethylacetate 1:1) to yield allyl-N-cinnamoyl-proline-leucine-isoleucinate as a crystalline solid, in good yields (68 %) $[\alpha]_D^{25} = -171^\circ$ ($c = 0.0075$, CH_2Cl_2).

^1H NMR, 400 MHz, CDCl_3 , δ 7.74 (d, $J = 15.4$ Hz, 1H), 7.55 - 7.50 (m, 2H), 7.39 - 7.35 (m, 3H), 6.84 (d, $J = 8.6$ Hz, 1H), 6.75 (d, $J = 15.4$ Hz, 1H), 5.90 (ddd, $J = 22.9$ Hz, 11.7 Hz & 5.8 Hz, 1H), 5.33 (dd, $J = 17.3$ Hz & 1.4 Hz, 1H), 5.24 (dd, $J = 10.5$ Hz, 1.2 Hz, 1H), 4.74 (d, $J = 6.1$ Hz, 1H), 4.63 (d, $J = 5.6$ Hz, 2H), 4.51 - 4.49 (m, 1H), 4.39 - 4.34 (m, 1H), 3.76 (d, $J = 5.6$ Hz, 1H), 3.66 (dd, $J = 16.6$ Hz & 9.3 Hz, 1H), 2.46 - 2.41 (m, 1H), 2.18 - 2.12 (m, 1H), 2.08 - 2.01 (m, 1H), 1.94 - 1.92 (m, 1H), 1.86 (bs, 1H), 1.76 (t, $J = 5.6$ Hz, 1H), 1.64 - 1.53 (m, 2H), 1.48 - 1.42 (m, 1H), 1.24 - 1.17 (m, 1H), 0.97 - 0.86 (m, 12H)

Synthesis of allyl-N-(3-phenylglycyl)-proline-leucine-isoleucinate (33)

To a solution of the allyl-N-cinnamoyl-proline-leucine-isoleucinate (766 mg, 1.5 mmol) in CH_3CN (7.5 mL) was added 2-methylpropanal (216 mg, 3 mmol) and PASCOS catalyst (~5 mg) and the contents were stirred under oxygen atmosphere at RT for 12 h. After this time, a fresh lot of the catalyst and 2-methylpropanal (216 mg, 3 mmol) were added to the reaction mixture and allowed to stir, until complete conversion of the olefin (TLC - R_f = 0.5; EtOAc:Hexane - 3:2). The catalyst was filtered off on a cindered funnel and acetonitrile was removed in vacuo. The resulting residue was taken in EtOAc (30 mL) and washed successively with saturated solution of NaHCO_3 (2X10 mL), water (2X10 mL) and brine (1X10 mL). Separating the organic phase, drying (Na_2SO_4) and concentration in vacuo, yielded the corresponding oxirane containing peptide **33** in high purity and yields (HPLC). This was further subjected to column chromatography (silica gel; EtOAc:Hexane - 1:1) for purification to get to the pure epoxide allyl-N-(3-phenylglycyl)-proline-leucine-isoleucinate **33** in good yields (66 %) $[\alpha]_D^{25} = -86.33^\circ$ ($c = 0.009$, CH_2Cl_2).

^1H NMR, 400 MHz, CDCl_3 , δ 7.66 (d, $J = 7.8$ Hz, 1H), 7.53 (m, 1H), 7.36 - 7.27 (m, 5H), 5.78 - 5.74 (m, 1H), 5.32 (d, $J = 17.3$ Hz, 1H), 5.22 (d, $J = 10.2$ Hz, 1H), 4.71 - 4.68 (m, 1H), 4.61 - 4.59 (m, 2H), 4.58 - 4.54 (m, 2H), 4.09 (s, 1H), 3.84 - 3.80 (m, 1H), 3.68 (s, 1H), 3.66 - 3.59 (m, 1H), 2.26 - 2.16 (m, 1H), 2.01 (dd, $J = 3.7$ Hz & 1.8 Hz, 1H), 1.99 - 1.92 (m, 4H), 1.68 - 1.62 (m, 3H), 1.49 - 1.40 (m, 1H), 1.24 (dt, $J = 9$ Hz, 1.9 Hz, 1H), 0.95 - 0.84 (m, 12H)

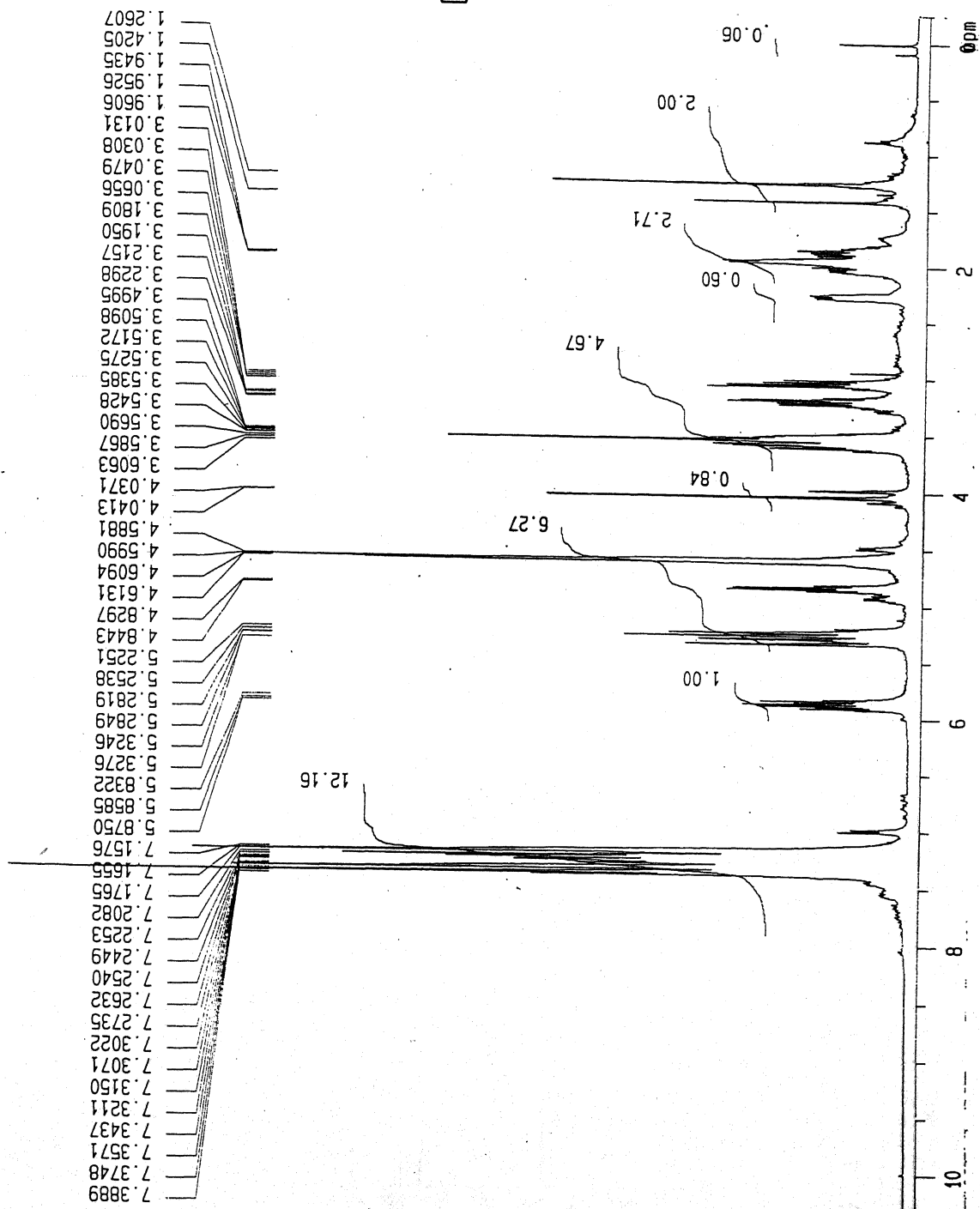
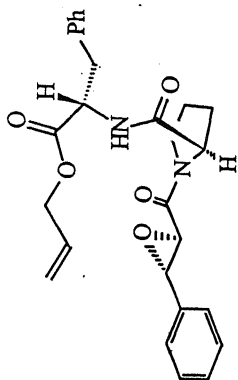
Synthesis of allyl-N,N-(allyl,*p*-methoxyphenyl)- β -phenylisoserine-proline-leucine-isoleucinate (34)

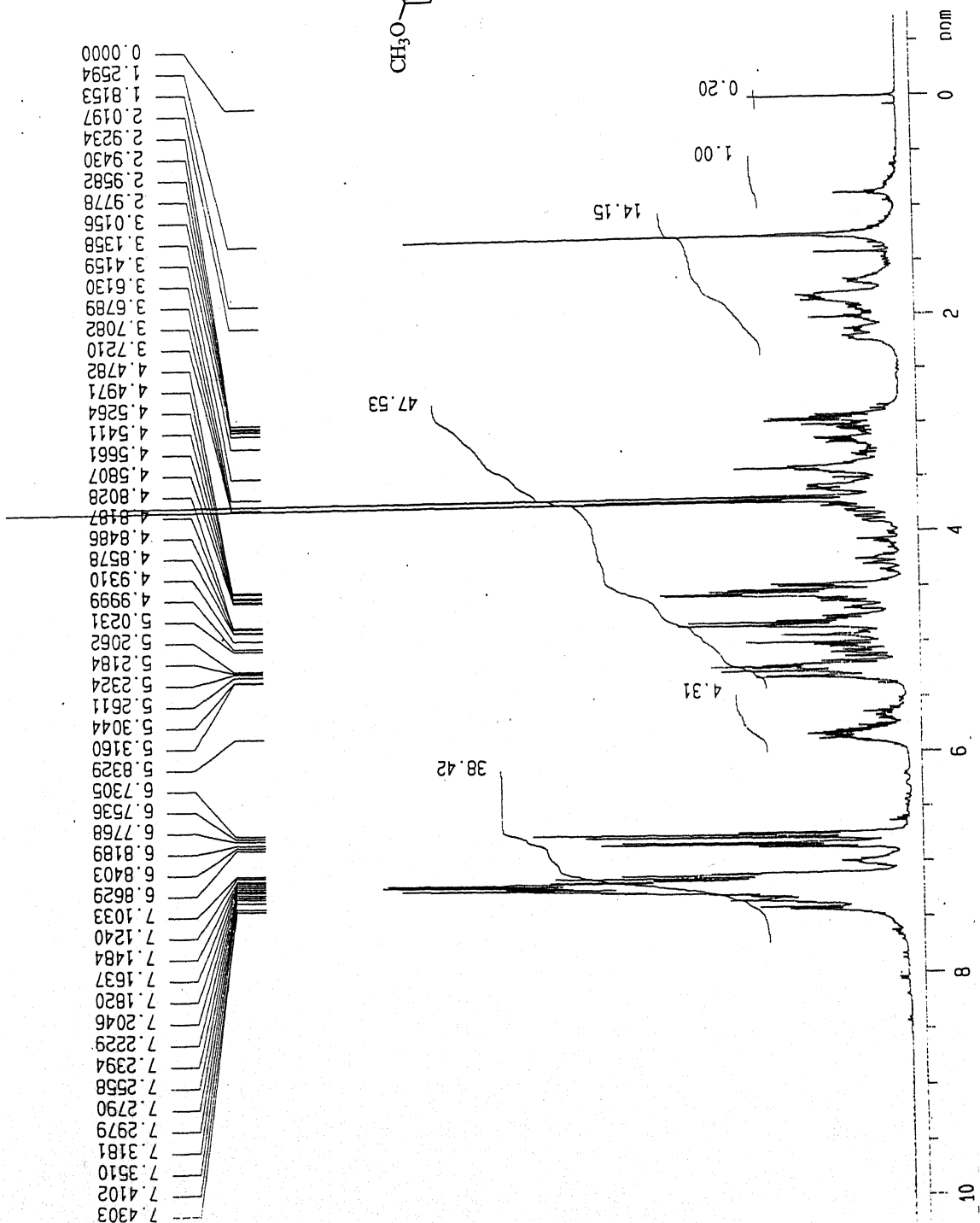
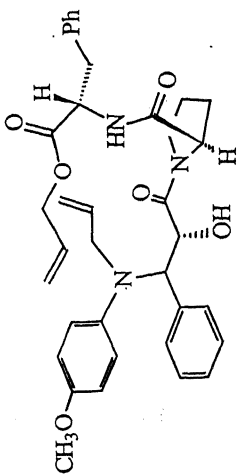
To a solution of allyl-N-(3-phenylglycyl)-proline-leucine-isoleucinate (633 mg, 1.2 mmol), in acetonitrile (7.5 mL) was added allyl-*p*-anisidine (196 mg, 1.2 mmol) and CoCl_2 (5 mg) and the contents were stirred until complete consumption of the epoxide (TLC - EtOAc:Hexane - 3:2). The solvent was removed under vacuum and the resulting residue was taken in EtOAc (20 mL) and washed with water (2X5 mL). The organic layer was separated, dried (Na_2SO_4) and concentrated in vacuo to yield a residue, which was subjected to purification by column chromatography (silica gel - EtOAc:hexane - 3:2) to yield allyl-N,N-(allyl,*p*-methoxyphenyl)- β -phenylisoserine-proline-leucine-isoleucinate **34**, in moderate yields (41 %) as a gum $[\alpha]_D^{25} = -63^\circ$ ($c = 0.0009$, CH_2Cl_2).

^1H NMR, CDCl_3 , 400 MHz, δ 7.65 (d, $J = 8.2$ Hz, 1H), 7.42 - 7.16 (m, 5H), 6.92 (d, $J = 8$ Hz, 1H), 6.80 (d, $J = 7.8$ Hz, 2H), 6.76 (d, $J = 7.8$ Hz, 2H), 5.97 - 5.83 (m, 2H), 6.34 (d, $J = 17.1$ Hz, 1H), 5.26 (d, $J = 10$ Hz, 1H), 5.18 (d, $J = 16.5$ Hz, 1H), 15.11 (d, $J = 10$ Hz, 1H), 4.99 (d, $J = 6.2$ Hz & 1.4 Hz, 1H), 4.89 (s, 1H), 4.63 (t, $J = 5.9$ Hz, 2H), 4.58 (dd, $J = 7.8$ Hz & 2.2 Hz, 2H), 4.50 - 4.48 (m, 1H), 4.39 (d, $J = 7.2$ Hz, 1H), 4.25 (dd, $J = 11.4$ Hz & 1.4 Hz, 1H), 3.73 (s, 3H), 3.67 - 3.68 (m, 1H), 3.52 - 3.48 (m, 1H), 2.16 (s, 1H), 2.04 - 1.81 (m, 3H), 1.67 - 1.60 (m, 1H), 1.50 - 1.39 (m, 1H), 1.25 (s, 1H), 0.91 (dd, $J = 9.5$ Hz & 5.4 Hz, 6H); MS m/z 691(M^+), 663, 578, 512, 381, 252, 211, 154; IR ν_{max} 3300(br), 3220(s), 3030, 2920, 2800, 1775, 1715, 1665, 1600, 1560, 730 cm^{-1}

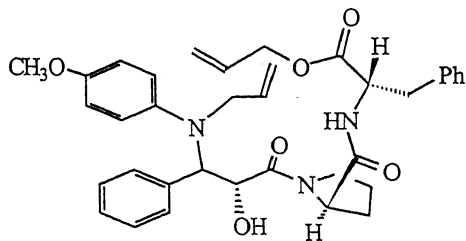
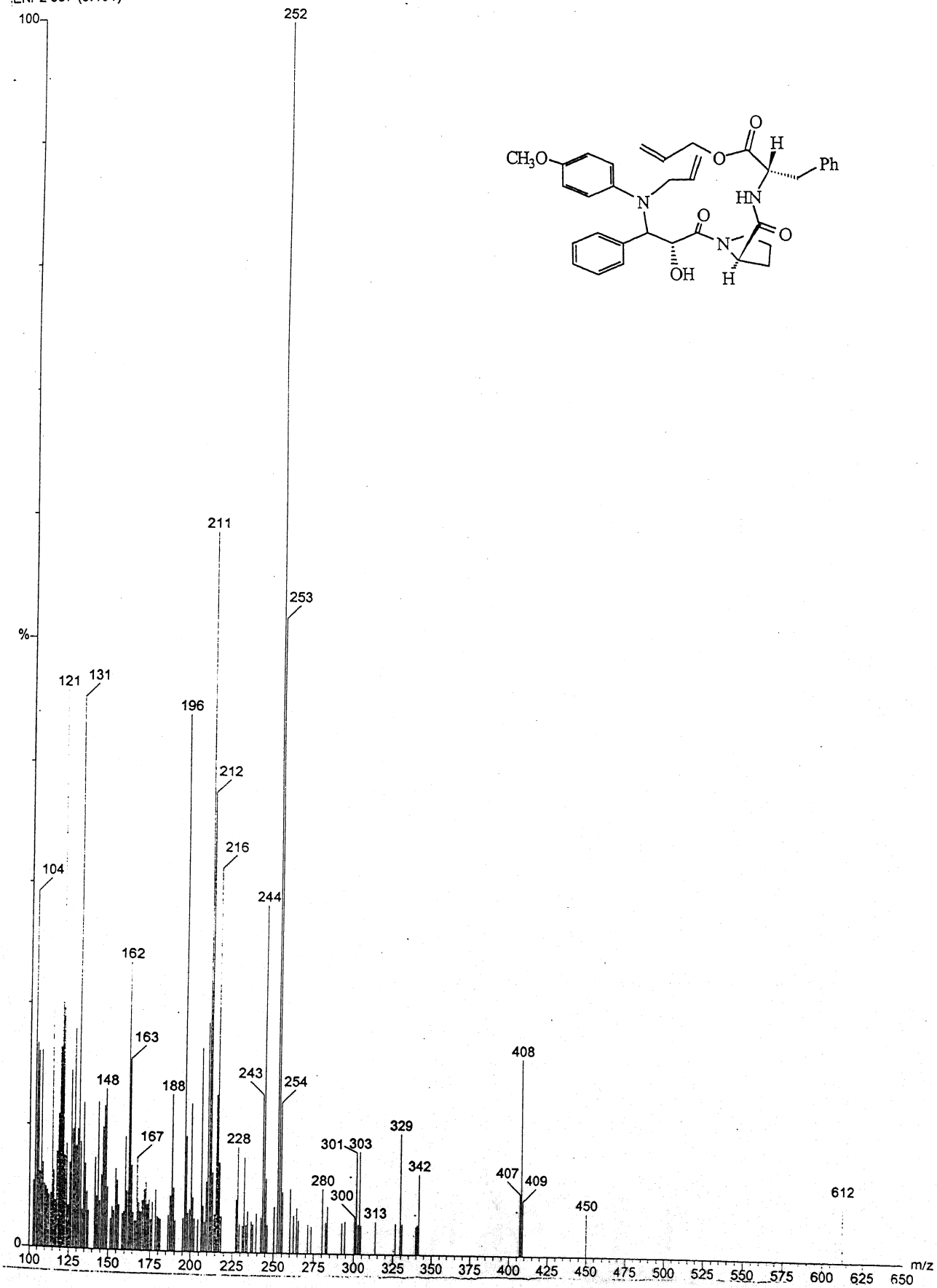
Attempts to synthesise the cyclic peptide (34) via RCM

To a clean dry flask flushed with N_2 , was added $(\text{Pcy})_3\text{Cl}_2\text{Ru}$ benzylidene catalyst (41 mg, 0.05 mmol) and CH_2Cl_2 (208 mL) and stirred for 15 min. at ambient conditions. A violet colored solution was formed. To this was added a solution of the diene allyl-N,N-(allyl,p-methoxyphenyl)- β -phenylisoserine-proline-leucine-isoleucinate **33** (172 mg, 0.25 mmol) in CH_2Cl_2 (208 mL), in a period of 1 h. through a syringe. The violet colored solution turned orange. The contents of the flask were stirred after complete addition of the diene **33** under reflux, in nitrogen atmosphere for 48 h. The reaction was quenched by stirring with 1 mL of water for 15 min. The organic phase was dried (Na_2SO_4), concentrated and subjected to flash column chromatography to isolate the starting material **33** as the major component of the mixture, along with the complete recovery of $\text{P}(\text{Cy})_3$. No traces of the expected cyclised product were found.



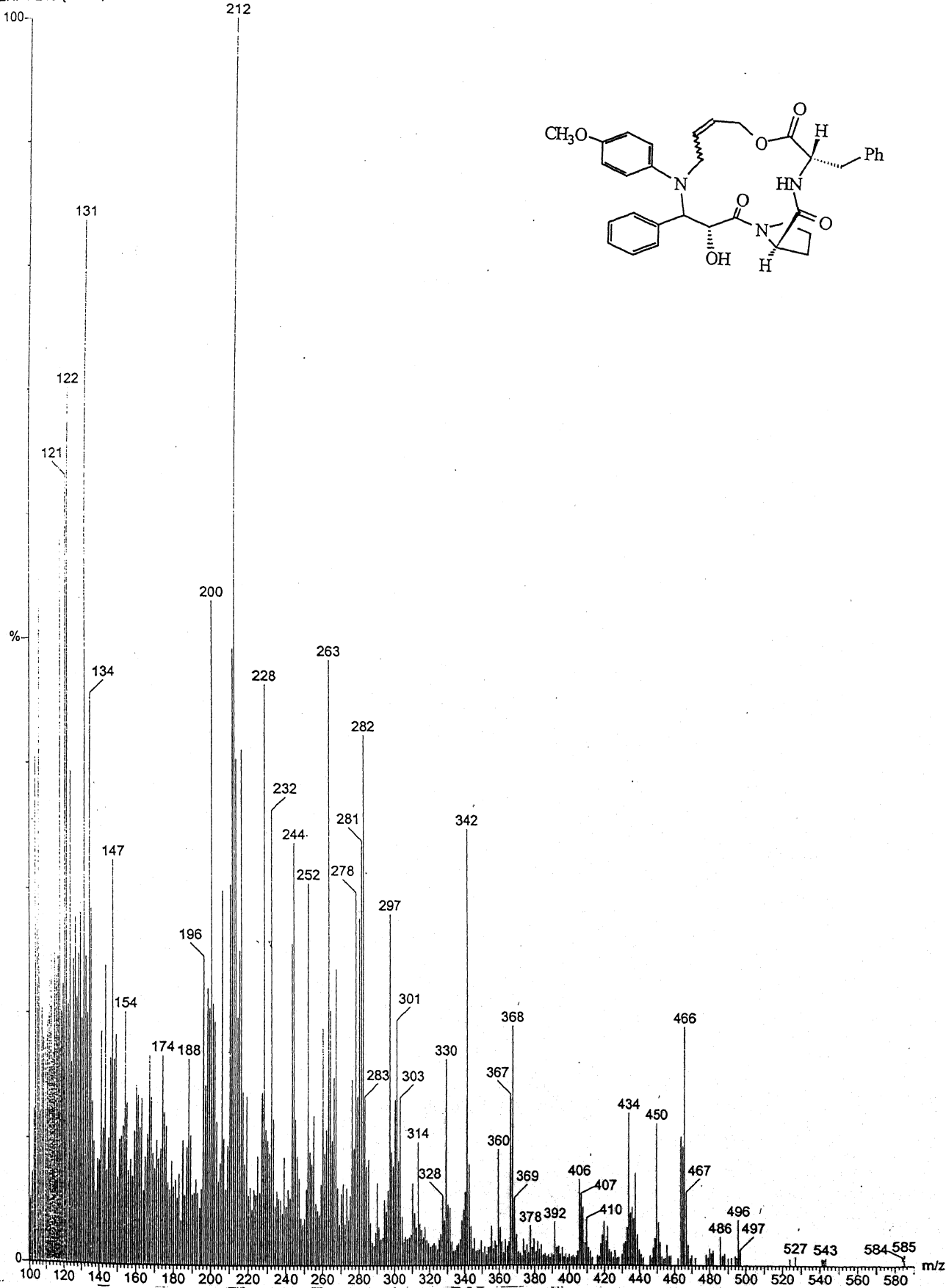
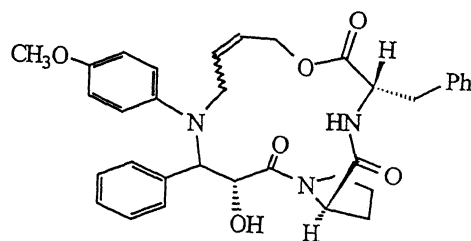


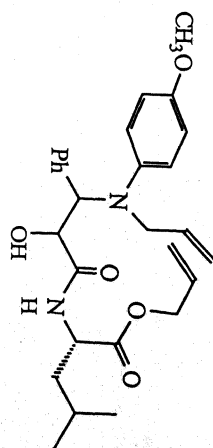
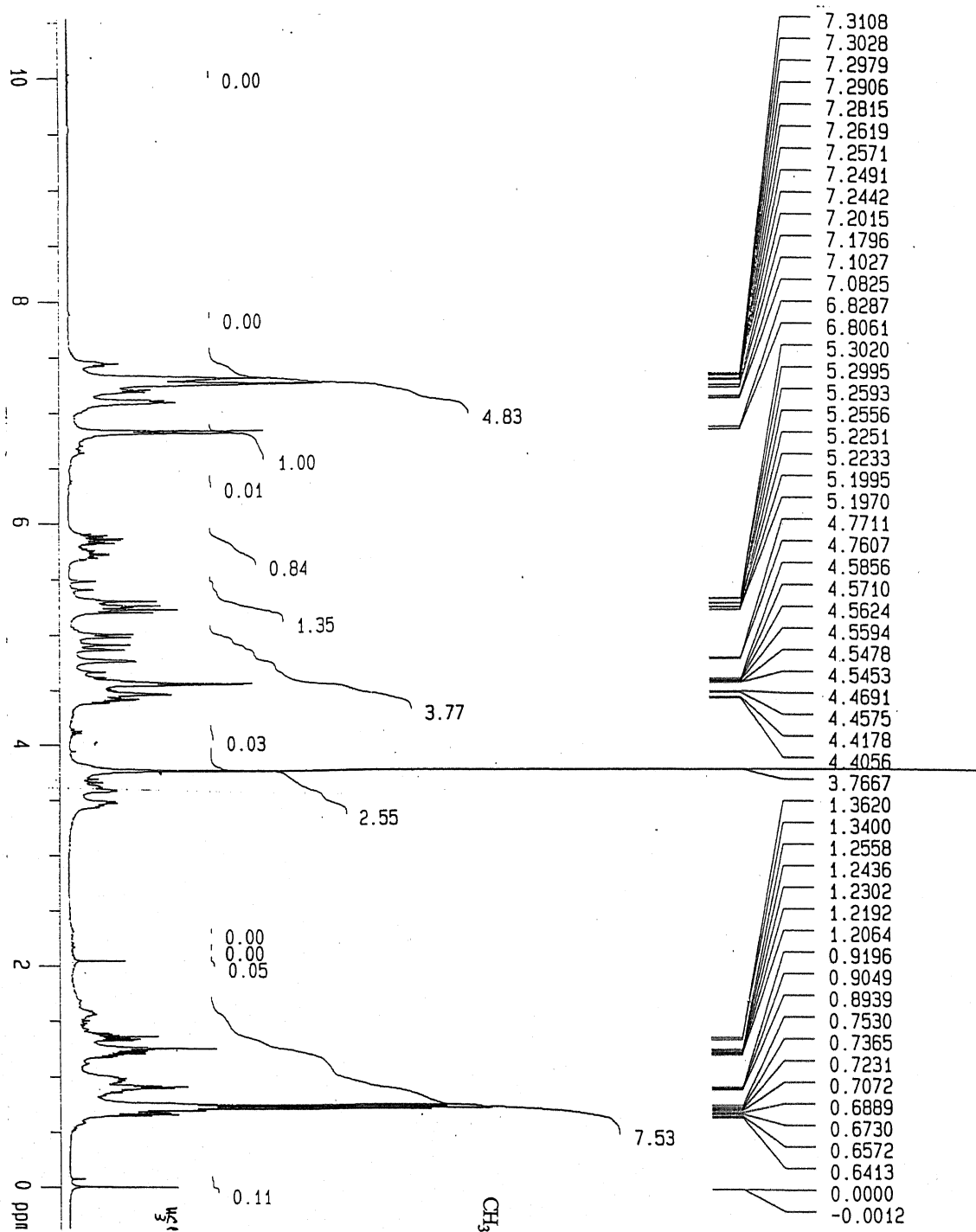
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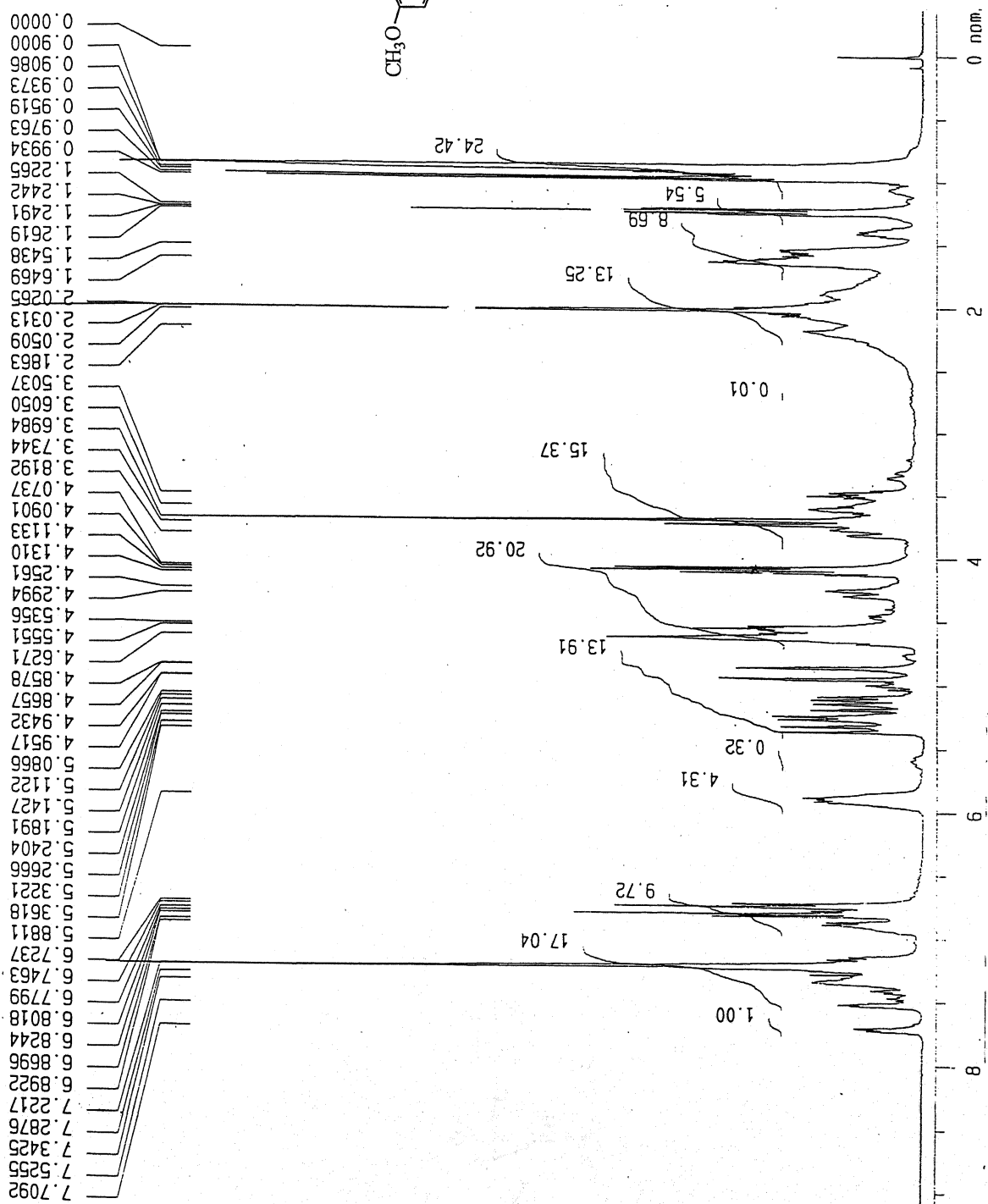
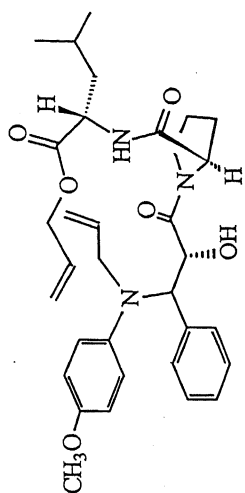


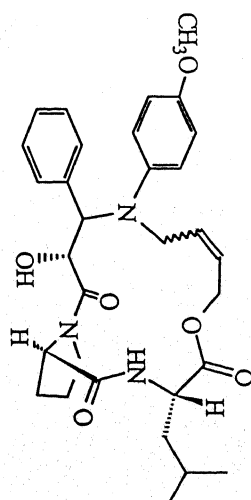
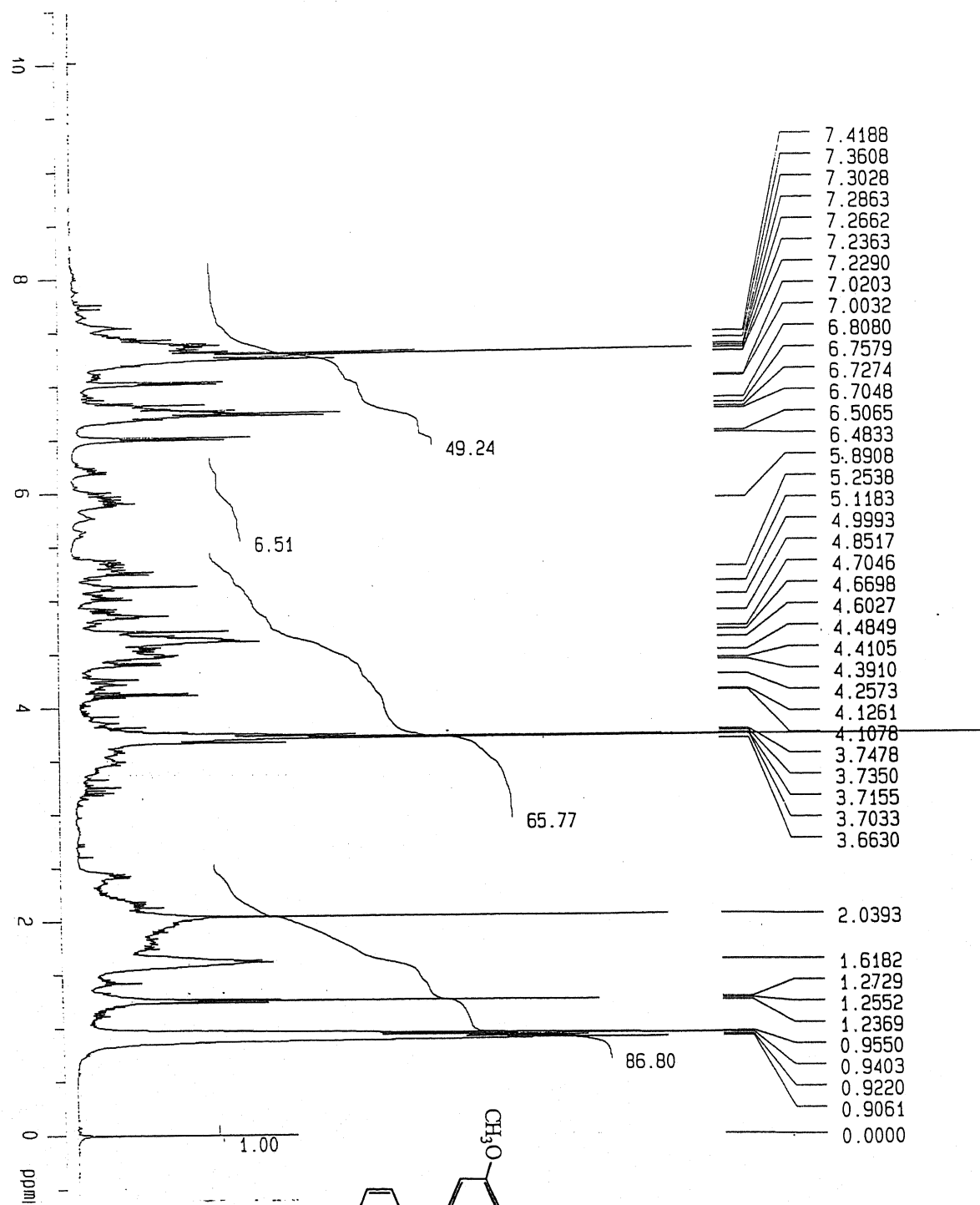
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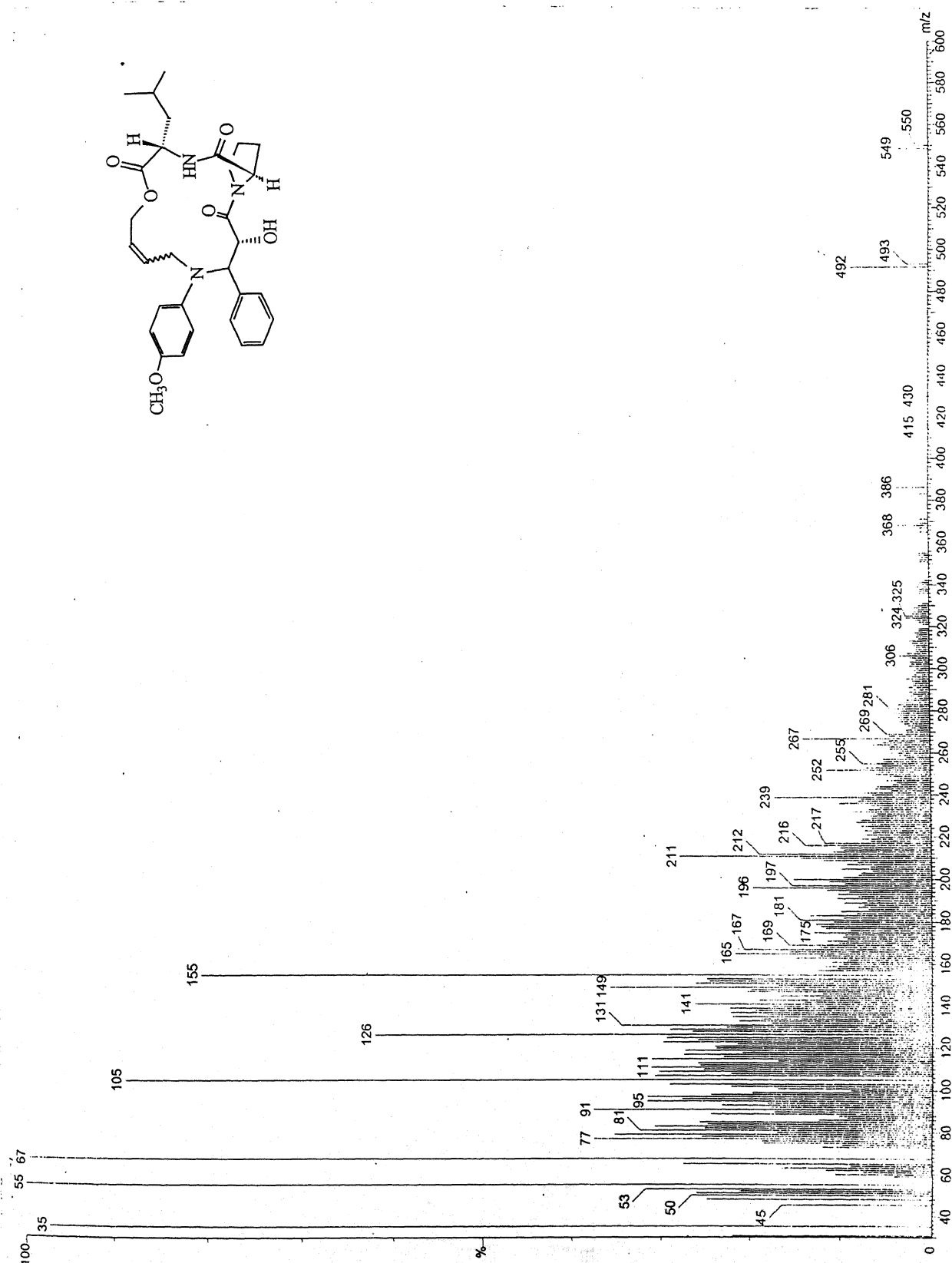
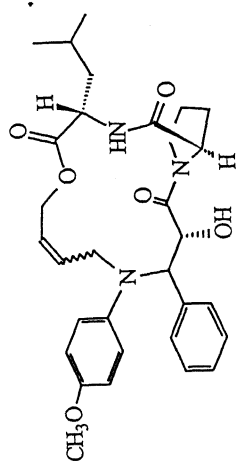
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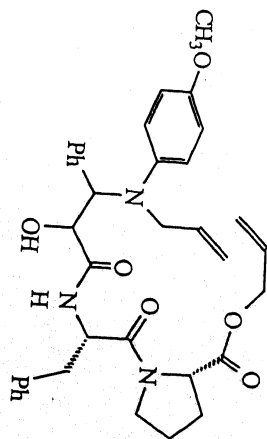
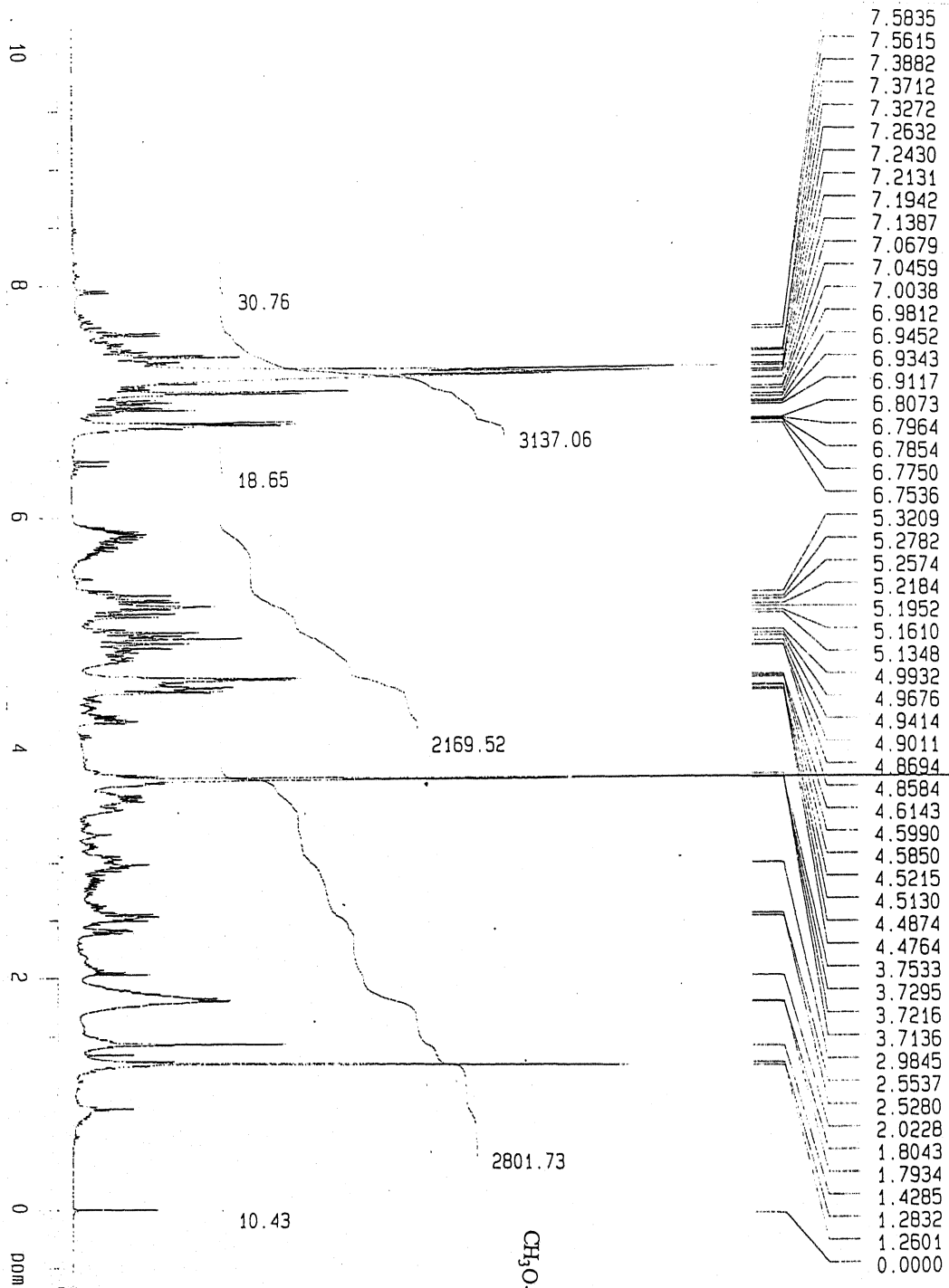


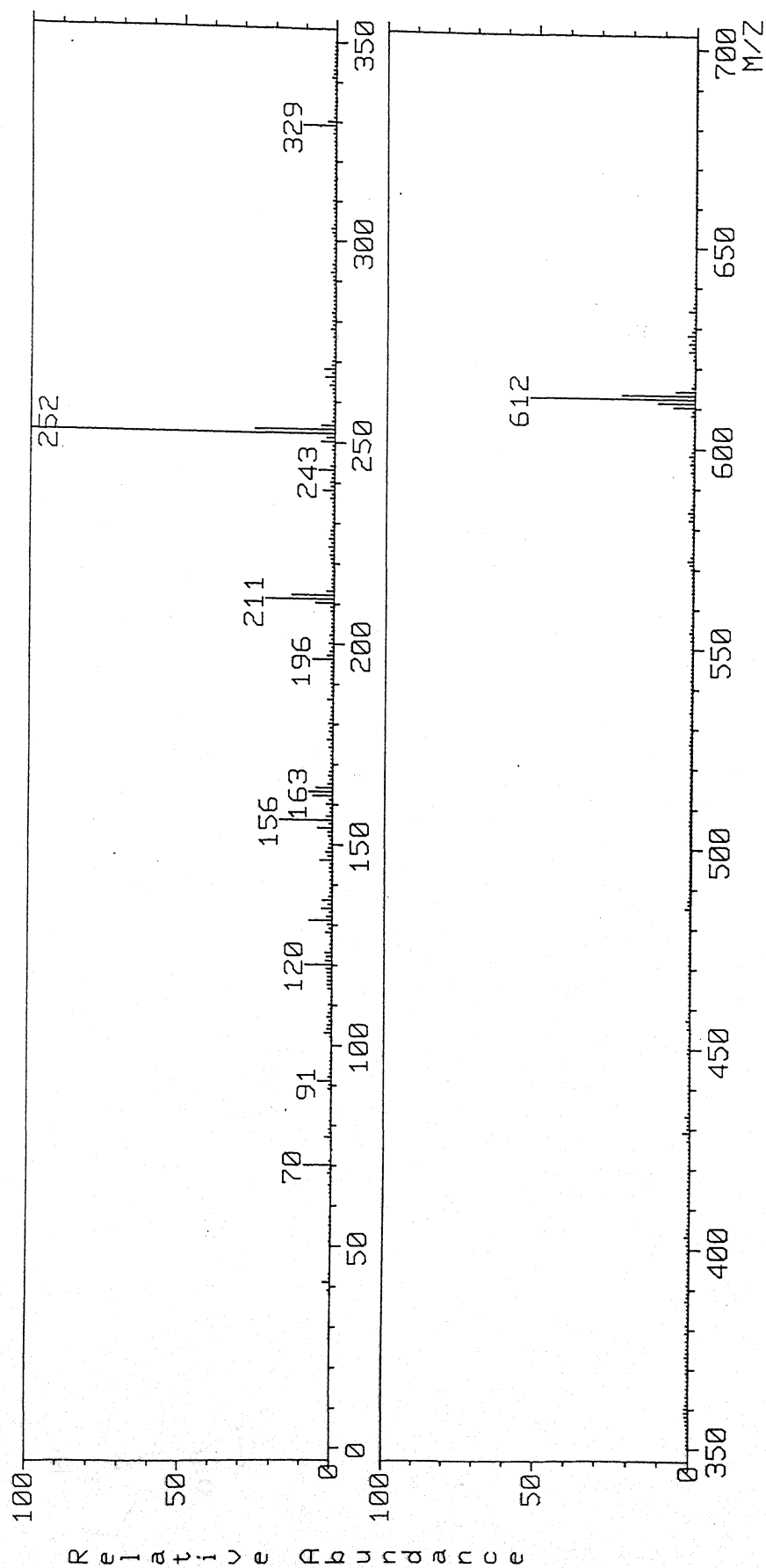
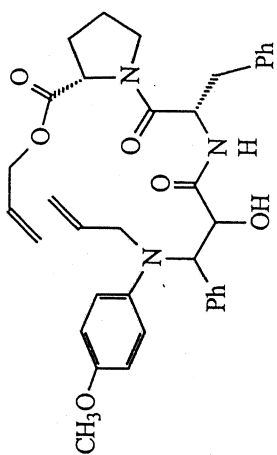


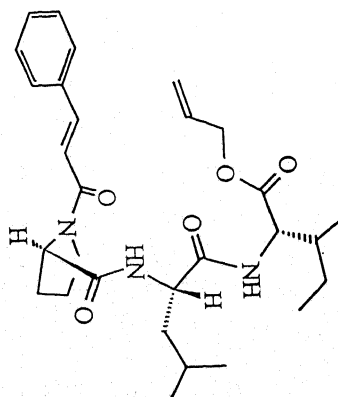
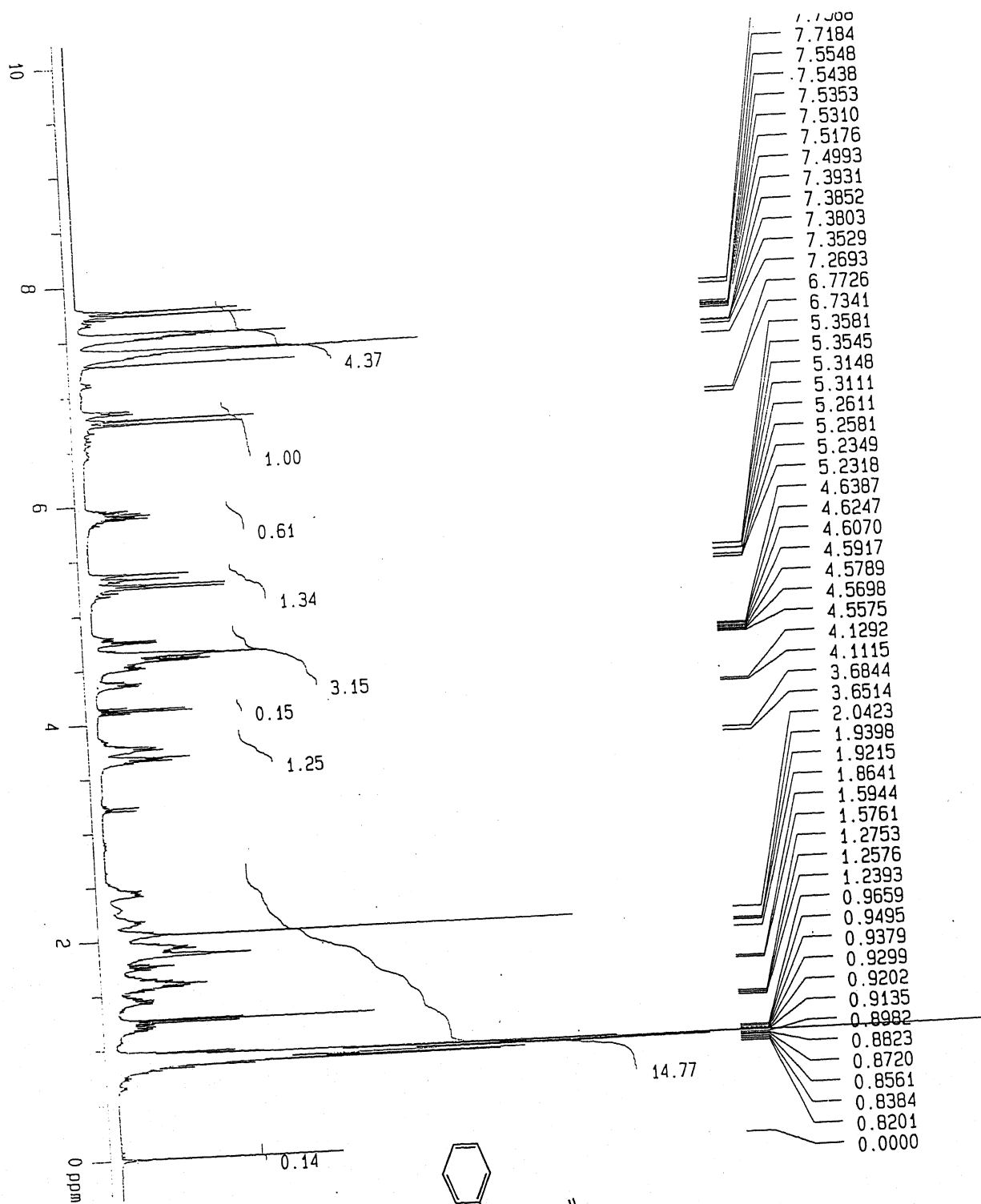


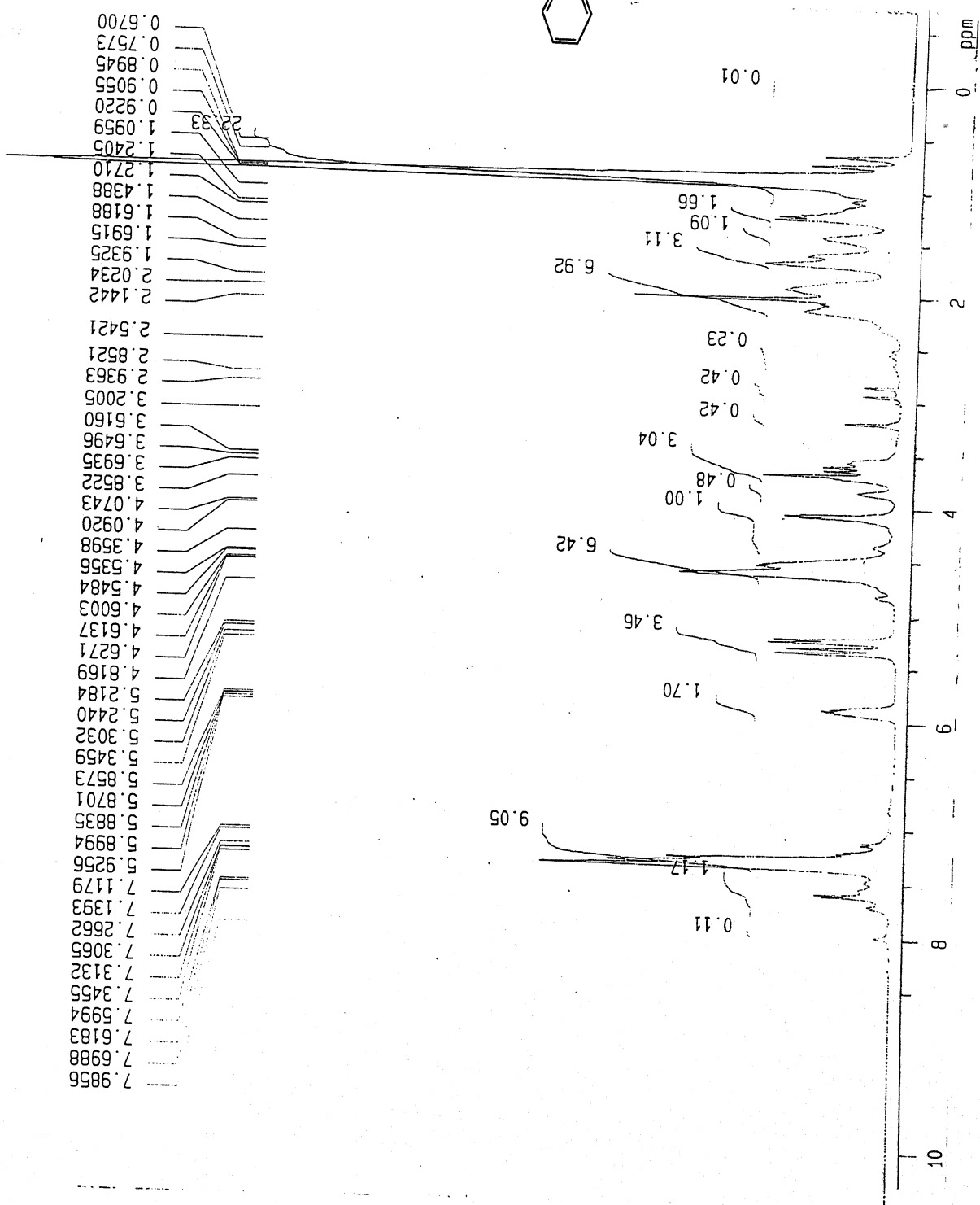
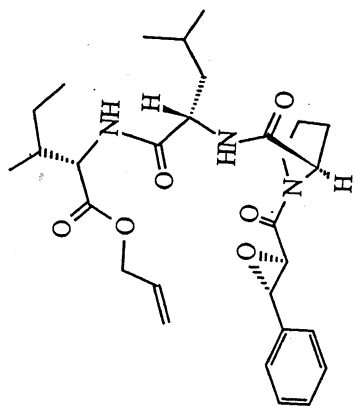


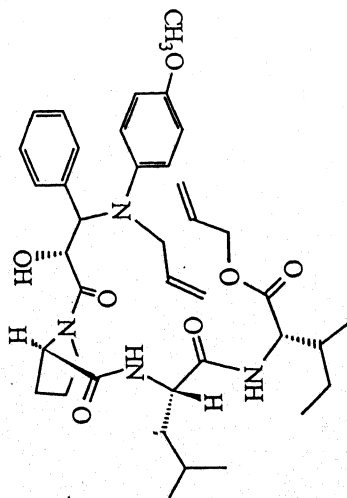
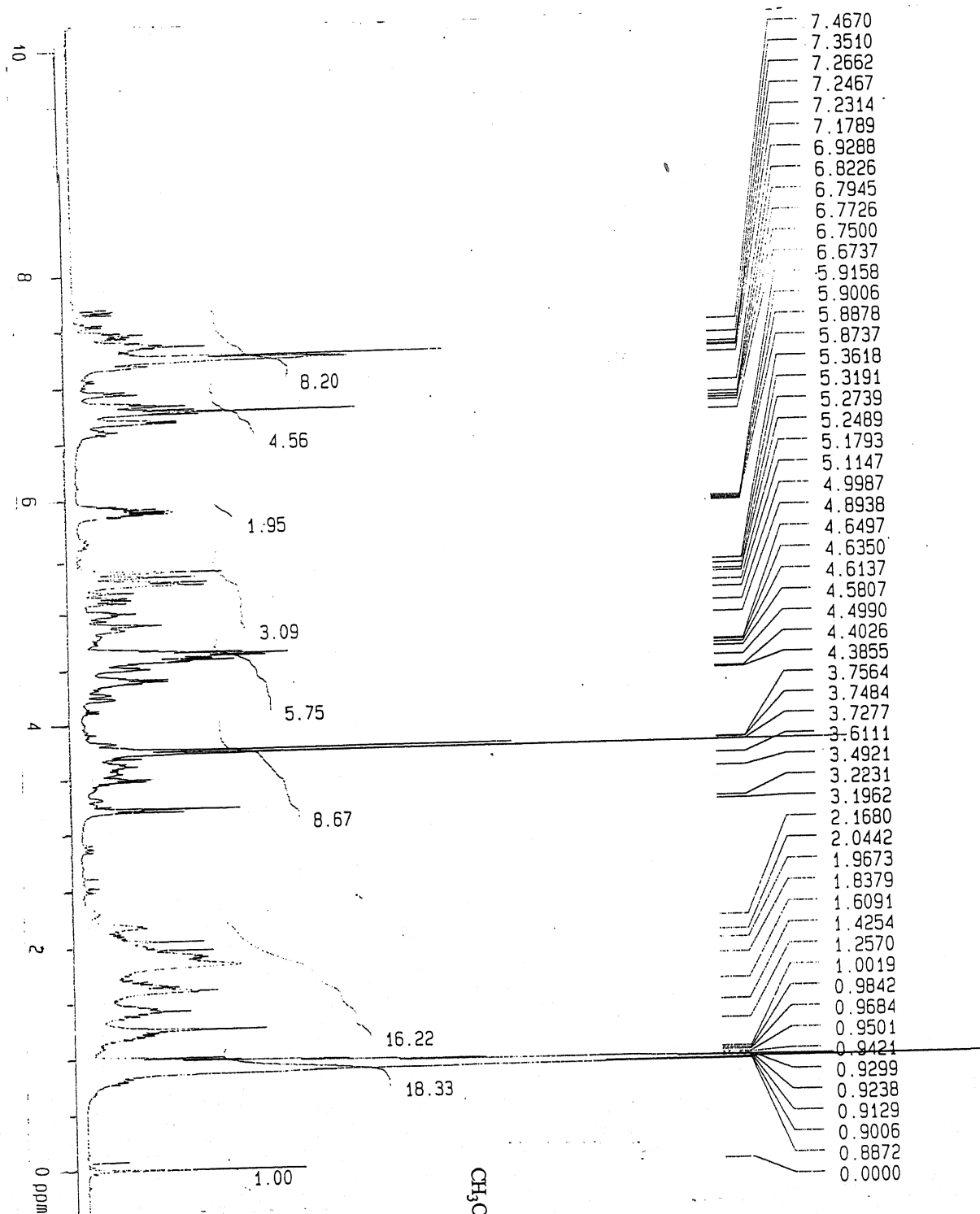


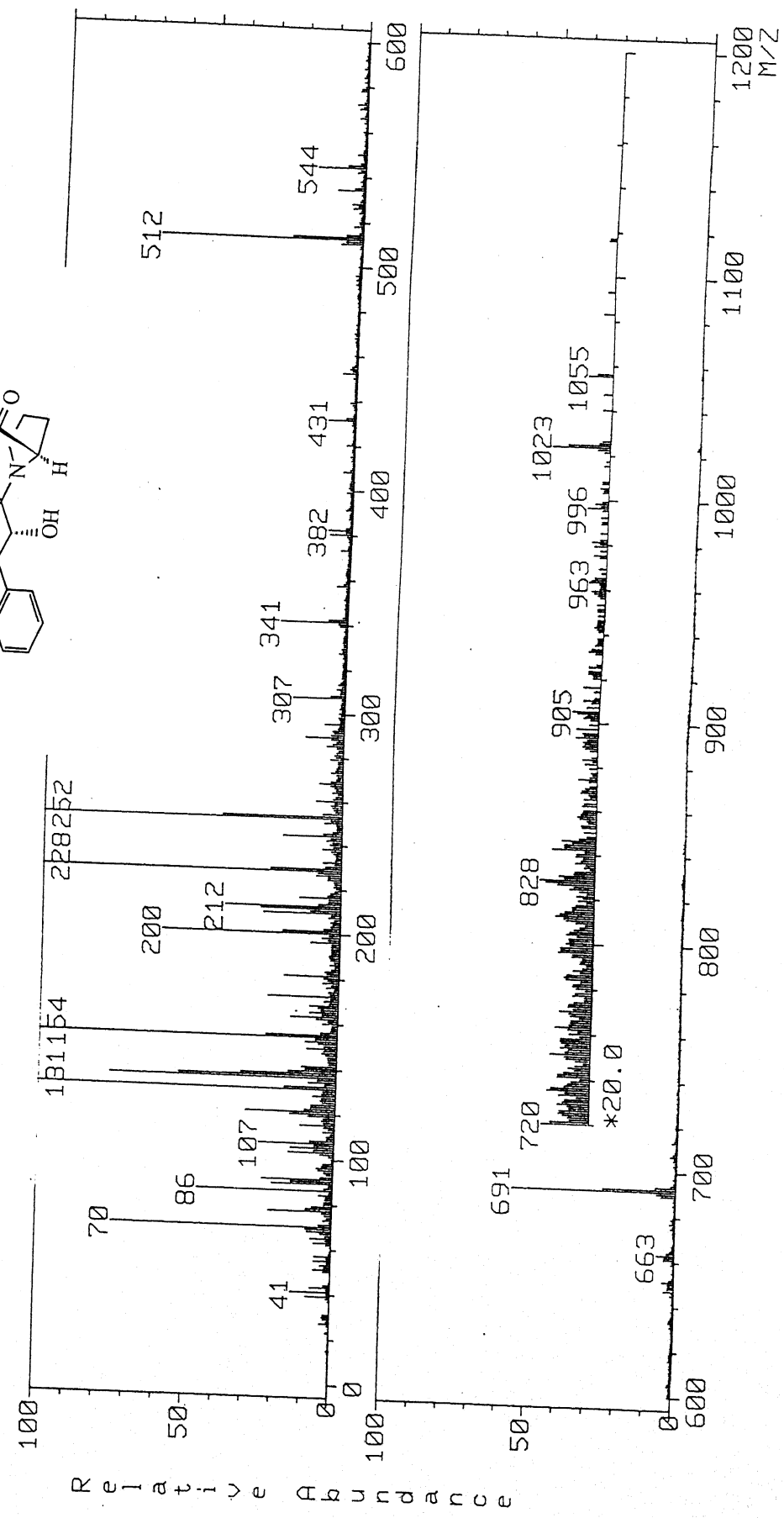
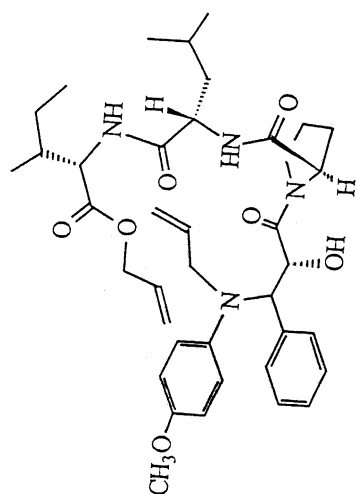


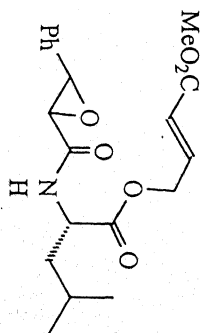
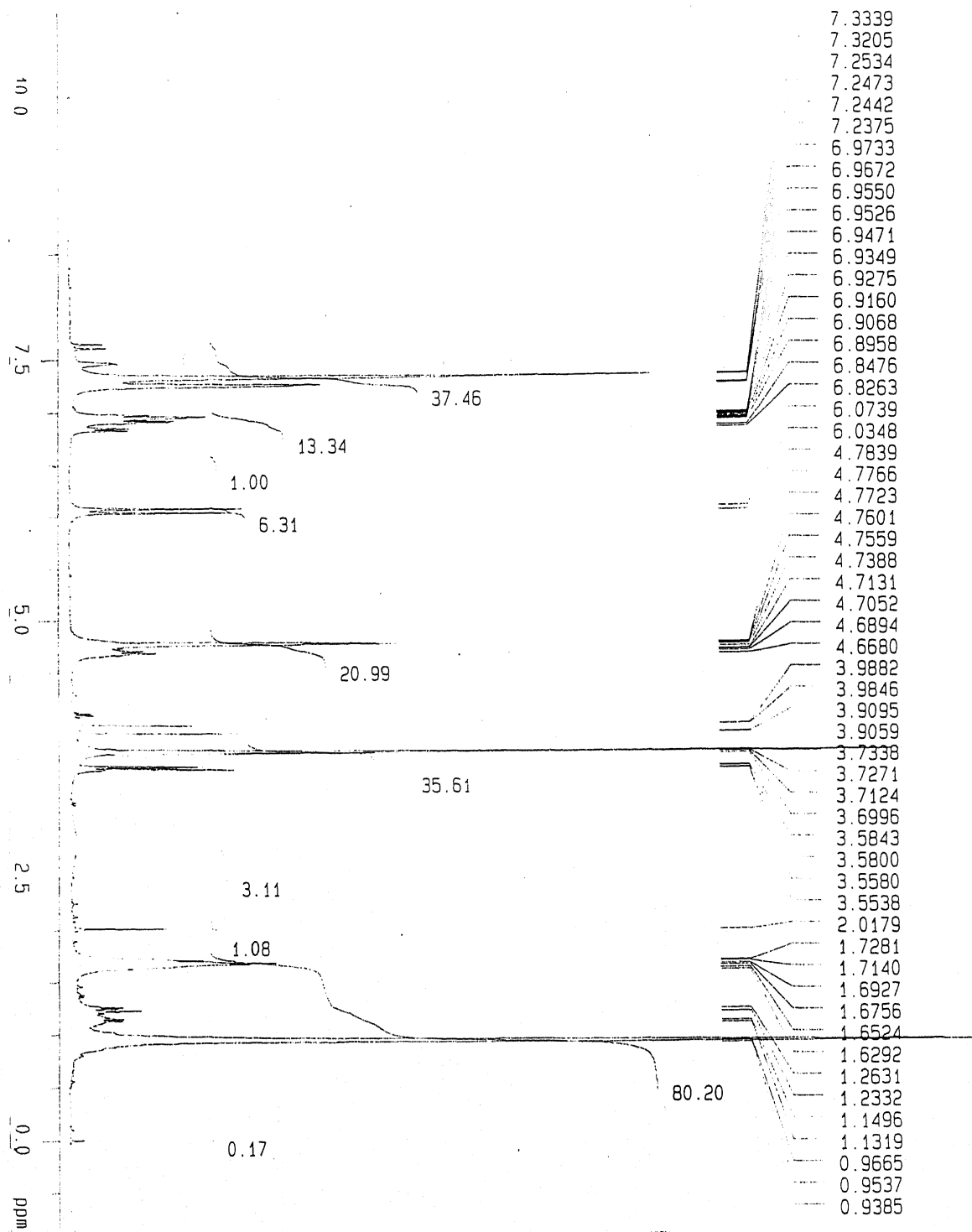


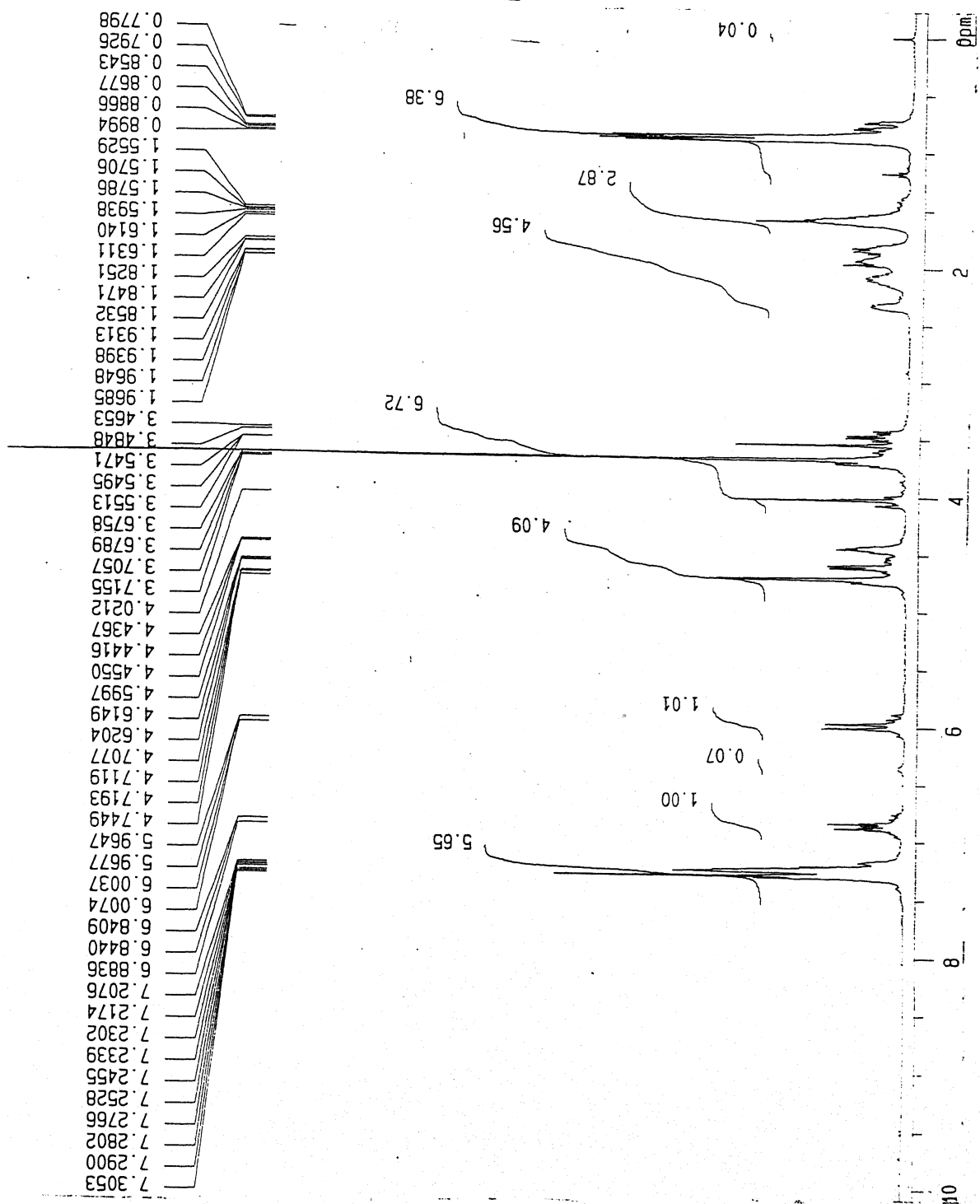
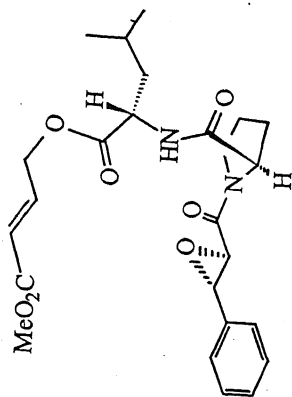


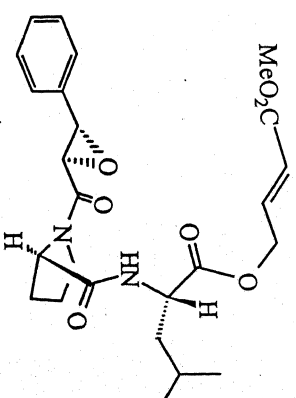
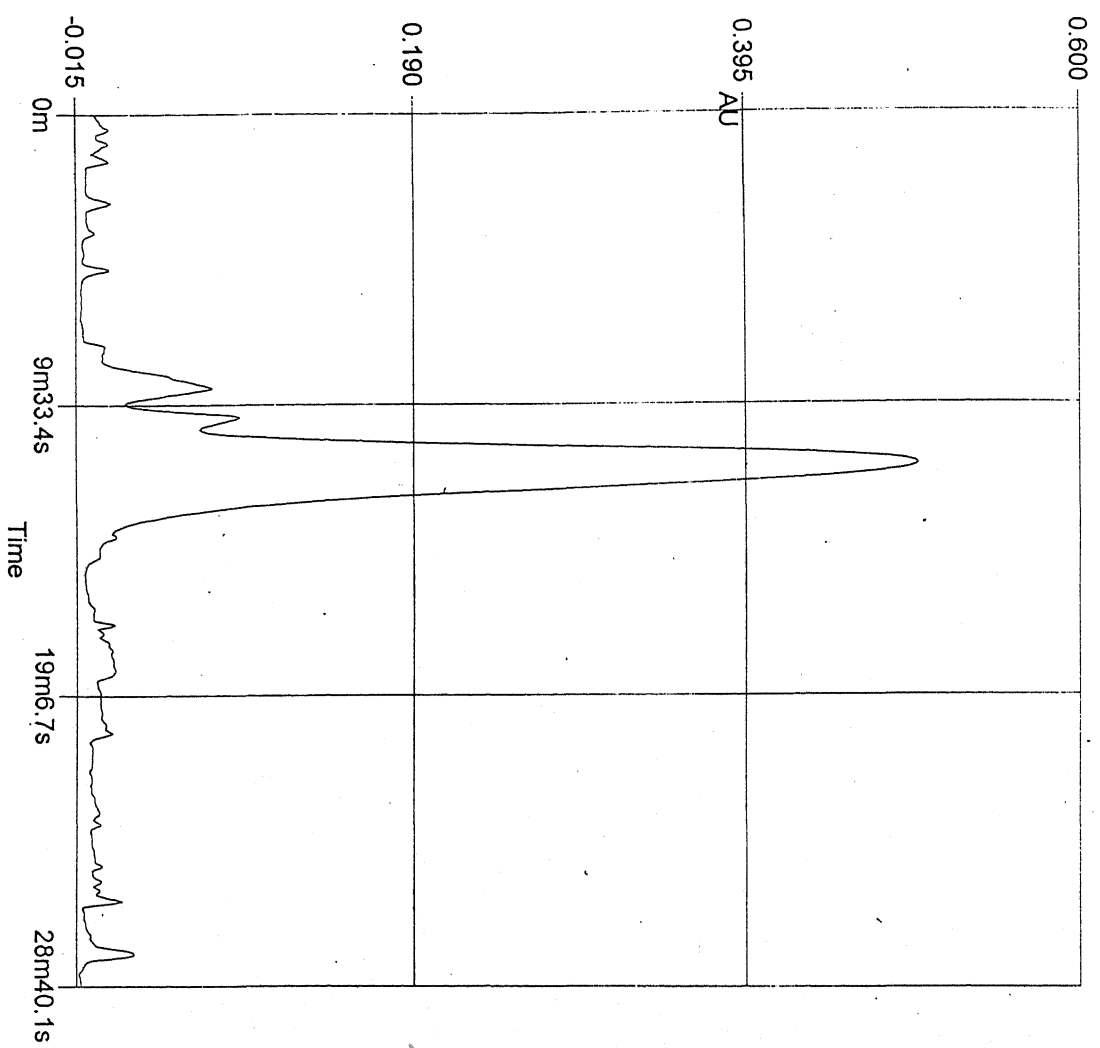






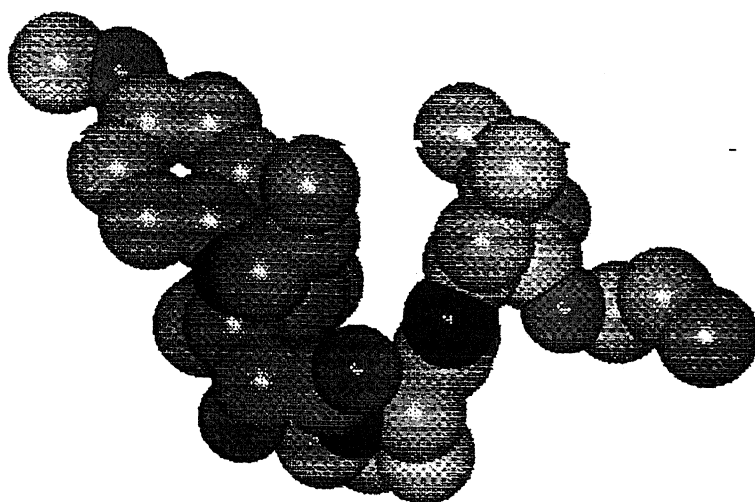




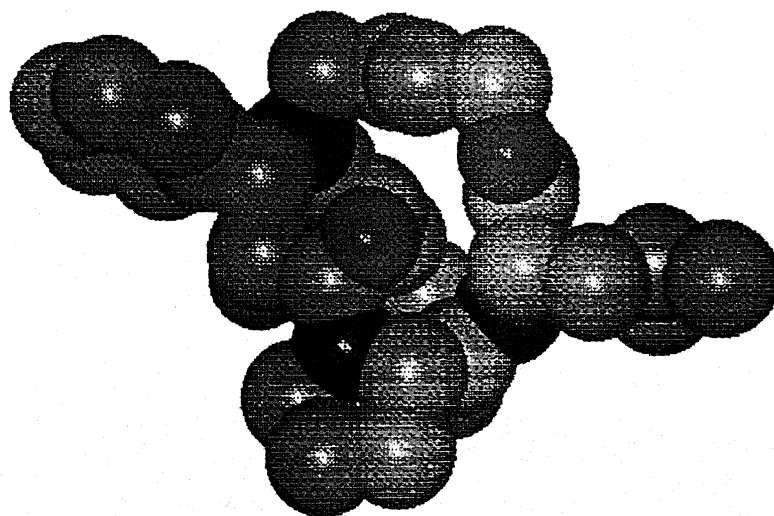


CHIRAL HPLC

Energy Minimized Structures of RCM Precursor 22a and The Cyclised Product 23a



γ -Turn in RCM PRECURSOR-22a



RCM CYCLISED PRODUCT - 23a

**PEPTIDES AS MIMICS FOR THE BIO-ACTIVE
CONFORMATION OF Phe-Pro RESIDUE OF HIV
PROTEASE LIGANDS**

Introduction

One of the exciting challenges in contemporary bio-organic chemistry is the determination of three-dimensional structural details of the complexes of biologically active peptides & pseudopeptides with enzyme active sites. This information may be exploited to design new ligands with even higher binding affinities for the enzyme binding site. Linear peptides exist in dynamic equilibrium in solution between a number of conformations differing little in energy. Knowledge of the preferred solution conformations of an oligopeptide does not necessarily provide information regarding the conformation of that ligand when bound to its respective host.³⁷² In the absence of three dimensional structural data for the ligand-receptor complex itself, information relating to the biologically active conformation of the ligand has been generally obtained by introducing conformational restraints by cyclisation or by incorporation of peptide mimics at selected sites of the peptide back bone.³⁷³⁻³⁷⁶ The resultant effects upon binding and biological activity of the pseudopeptides are then evaluated in connection with the available reduced conformational space to provide insight into the biologically active conformation of the ligand. This approach reflects the accepted view that biologically active peptides adopt discrete conformations upon binding, even though many energetically similar conformations are accessible.³⁷⁷

Recently, there have been several reports of peptide mimics that enforce extended structure without making any provision for orienting the side chains.³⁷⁸ The importance of controlling the side chain orientations of pseudopeptides has been recognised because these appendages provide crucial sites for recognition, specificity, binding, and consequently transduction.³⁷⁹

Constrained amino acids have been utilized to restrict the amino acid conformation and hence that of the backbone carrying it, in the design of numerous inhibitors of HIV PR.

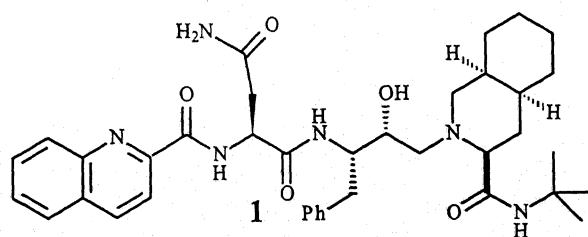
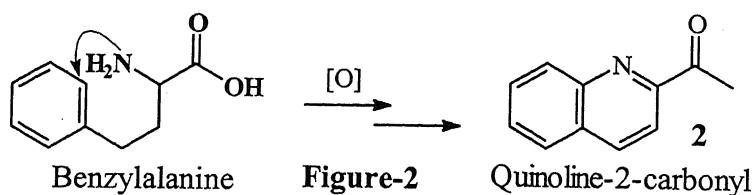


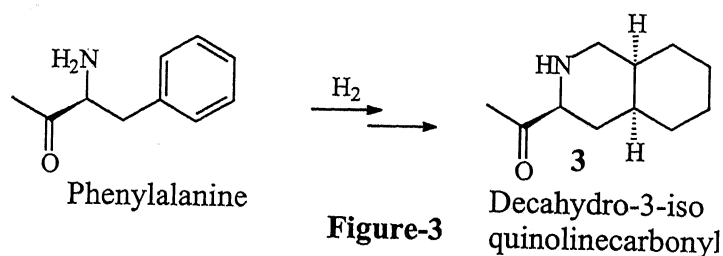
Figure-1 R₀ 31-8959 SAQUINAVIR

Saquinavir (Ro 31-8959),⁹⁷ is one of the most potent inhibitors known to date, of HIV PR. It incorporates two novel amino acids, which can be considered as the constrained

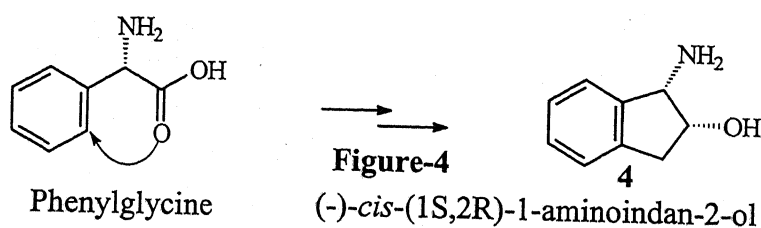
versions of benzylalanine and phenylalanine, at the P2 and P1' sites respectively. The P2 site quinoline-2-carbonyl, can be considered to be formed by covalently connecting the



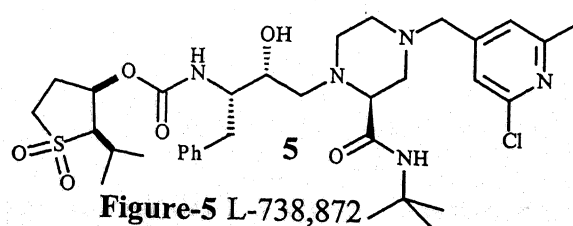
nitrogen of benzylalanine to the phenyl *ortho*-carbon and complete aromatisation of the resulting bicycle, acts as the cyclised benzylalanine analogue; on the other hand, complete



reduction, of similarly cyclised phenylalanine with a CH₂ linker, results in DIQ [(4a*S*,8a*S*)-decahydro-3(*S*)-isoquinolinecarbonyl] which has proved to be an excellent surrogate for proline at the P1' site.



Hydroxyethylene dipeptide isosteres (HDI) bearing the cyclic phenylglycine surrogate (-)-*cis*-(1*S*,2*R*)-1-aminoindan-2-ol have been demonstrated⁶³ to be potent and selective inhibitors of HIV protease.



One of the most potent, orally-available inhibitors of HIV PR L-738,872,⁹⁹ (Figure-5) which is currently under clinical trial for the treatment of HIV infection, incorporating a

hydroxyethylamine isostere contains cyclic sulphone at the P_2 site. The 2-isopropyl moiety (Figure-6) imparts a local constrain on the ligand, at the P_2 site.

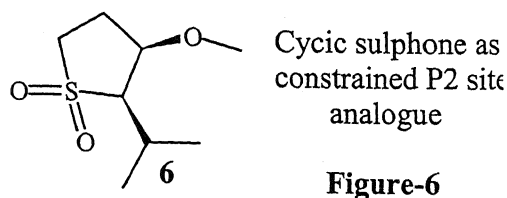


Figure-6

The 2-hydroxy-tetrahydrofuran moiety at the P_2 site of VX-478,¹⁰⁰ another potent HIV PR inhibitor, can be considered as a constrained analogue of an ethyleneglycol isostere at the P_2 site.

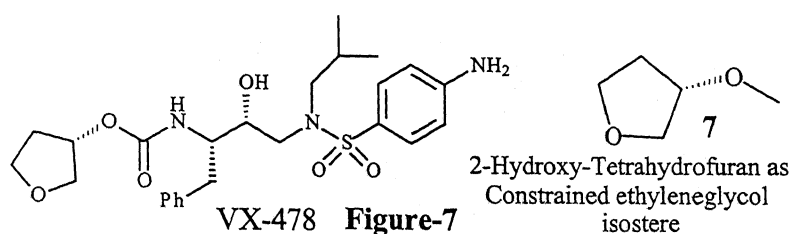


Figure-7

This can be better realised through the successful design of a potent, ritonavir analogue^{70,68} containing furofuran as the constrained P_2 ligand. X-ray crystal structural studies of protease bound inhibitor complexes, showed that each of the two oxygen atoms of the furofuran hydrogen bonds to the NH groups of the Asp29 and Asp30 respectively, of the viral protease.⁷⁰

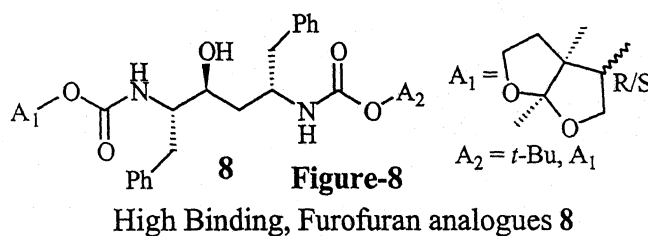
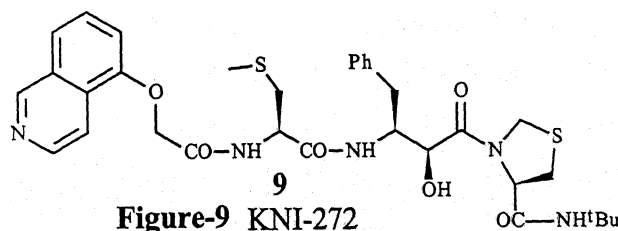


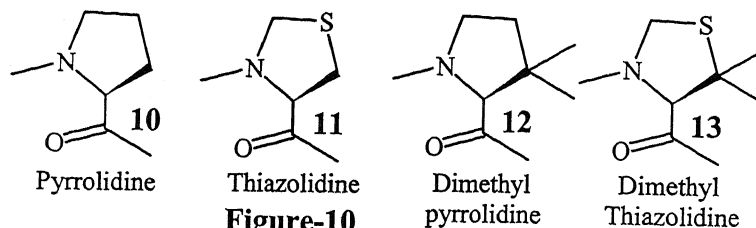
Figure-8

Kynostatins (KNIs),²⁴⁰ one of the few class of inhibitors that exhibit picomolar potencies



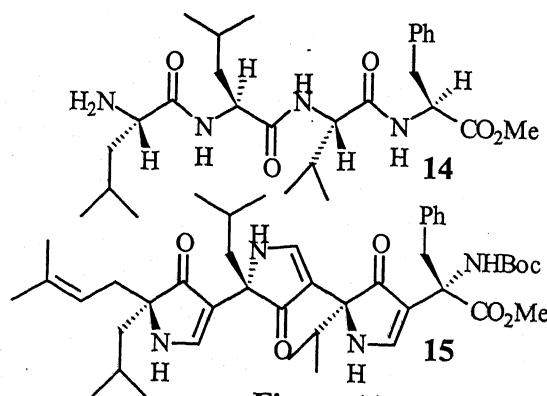
against HIV PR, incorporate a constrained proline analogue, the thiazolidine (KNI-125), dimethylpyrrolidine (KNI-125) or dimethylthiazolidine (KNI-162), in place of the P_1 proline residue (figure-10).

Such an introduction has resulted in the enhancement of protease inhibitory activity.²⁴¹



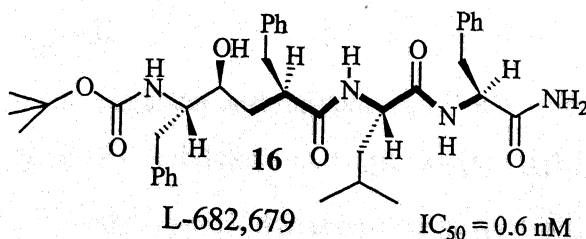
P1'pyrrolidine replacements in Kynostatins

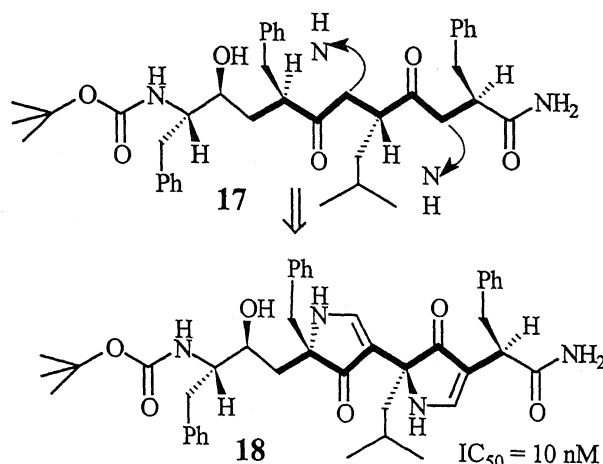
Pyrrolinone based peptidomimetics, the first mimics of β -strands, have been shown³⁷⁷ to be excellent inhibitors of HIV PR. Smith *et al.* have reported³⁷⁷ that pyrrolinone inhibitors offer better transport properties than the corresponding peptide based peptidomimetics. Structure-activity relationships for the pyrrolinones also correlated well with those reported for related peptides, consistent with similar modes of binding. The bis(pyrrolinones) have been shown to be more active in cellular antiviral assays compared with the analogous peptide-derived inhibitors. Novel, linked 3,5,5-pyrrolin-4-one scaffolding **15** (a tris(pyrrolinone)) has been shown to entirely replace the native peptide backbone⁷⁴ of the tetra-peptide equine angiotensinogen fragment **14**.



Tetrapeptide equine angiotensinogen fragment **14**;
structure of (β -strand) tris(pyrrolidinone) peptidomimetic

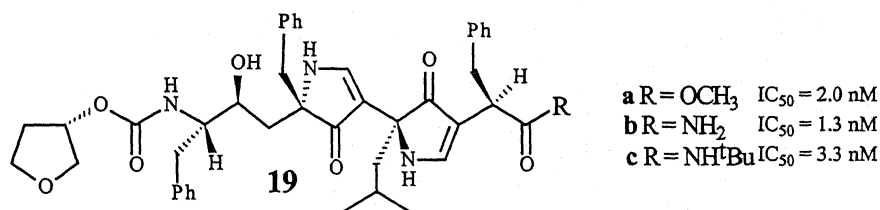
In the design of another pyrrolinone containing inhibitor,³⁷⁷ the P_1' - P_3' tripeptide sequence of L-682,679, a potent inhibitor of HIV PR was replaced by a bis(pyrrolinone) bearing the three requisite side chains.



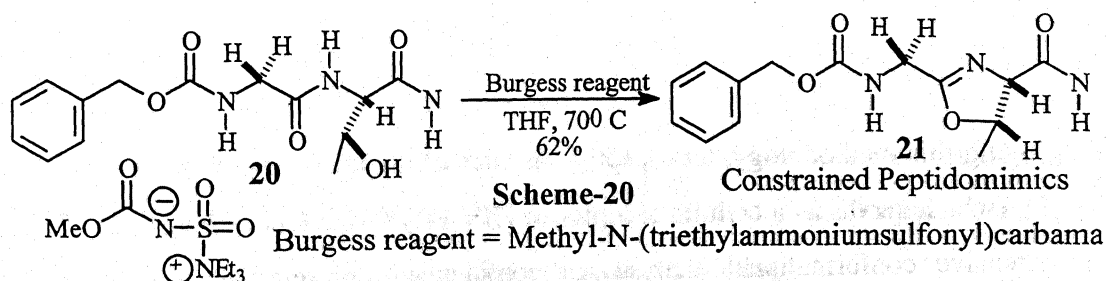
**Figure-12**

Merck inhibitor L-682,679 as the model for a prototypic pyrrolidine-based inhibitor **3**. Pyrrolinone motif is generated by NH displacement from peptide backbone

A schematic representation of the design of the bis(pyrrolinone) analogue of L-682,679, has been presented above. The pyrrolinone motif is generated by NH displacement from peptide backbone. Lead optimisation studies resulted in tetrahydrofuran as the best P_2 residue for high anti-viral activity. A few potent inhibitors, incorporating the bis (pyrrolinone) moiety, with different P_3' site extensions have been reported to be highly potent inhibitors of HIV PR.

**Figure-13 Analogues of Bis(pyrrolinone) 19**

Dihydrooxazoles have been found to be good constrained analogues of peptides.¹³³ A direct preparation of 4,5-dihydrooxazoles **21** from β -hydroxy- α -amino acids **20** has been reported by the cyclisation of serine and threonine derivatives with Burgess reagent in



one-step.

Pillai *et al* have described the design and synthesis of non-peptidic compounds that feature rigid backbone conformations and present various side-chain functions.¹³⁴ A dipeptide of interest (Figure 15a) is mimicked with a bicyclic vinylamide (Figure 15b) which arises from the condensation of a ketone with an aspartic acid derivative (Figure 15c).

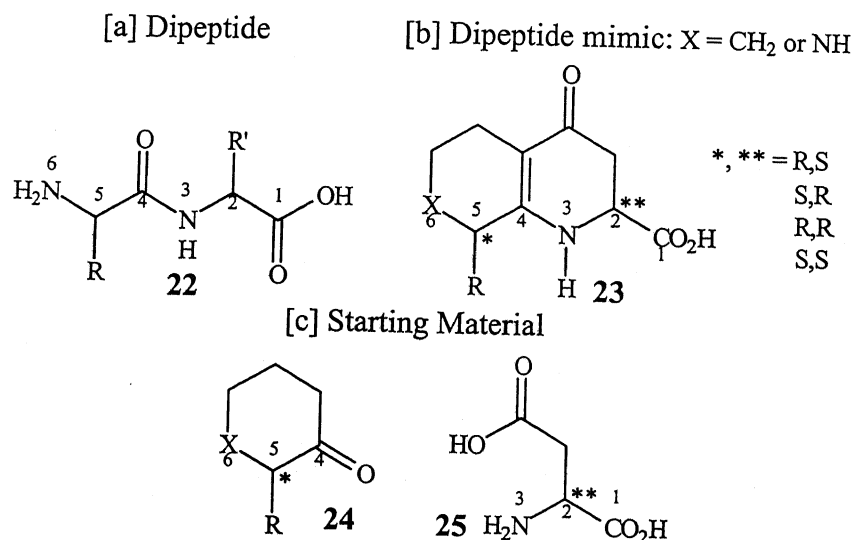


Figure-14 General approach by Pillai *et al*.

A comparison of the dipeptide 22 and its mimic 23 shows that C₂ and X₆ are used to make constraints in a standard fashion while C₄ is utilised as part of a vinylamide moiety. The C₅ side chain (R) originates from the ketone starting material whereas a second side chain (R') could arise from either the use of an aspartic acid derivative, which contains this group at C₂, or from attaching a similar group at a later stage with the C₁ carboxylate.

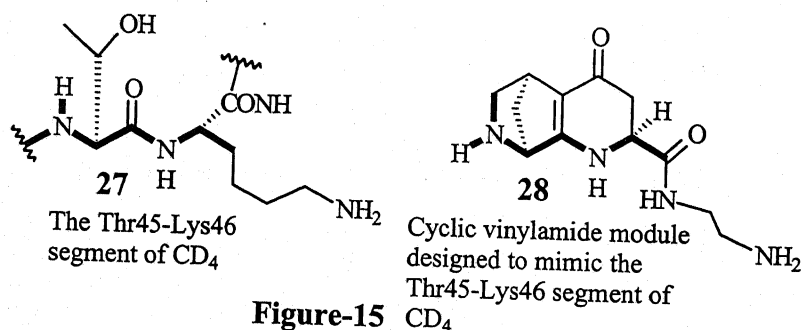
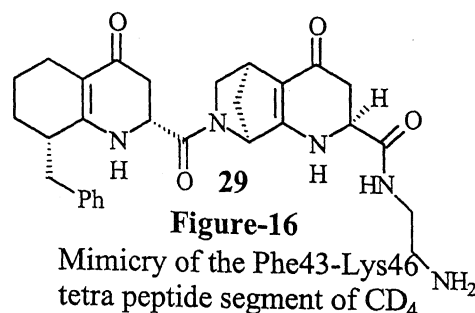


Figure-15

Employing this methodology, tetra-peptide mimics of the tetra peptide β -strand segment of CD₄ (which serves as a cellular receptor of HIV PR)³⁸⁰ have been constructed. Based on extensive conformational analyses of peptides and protein systems involved in proteolytic processing, which showed that cleavage sites are often located adjacent to

turns,¹⁶⁸ β -turn mimics in the form of constrained amino acids have been introduced at the P2 site of structural analogues of potent HIV PR inhibitors.²⁴²



Martin *et al* have reported⁹⁶ on the basis of molecular modelling studies that 1,2,3-trisubstituted cyclopropanes constitute novel rigid isosteric replacements of an extended conformation of the corresponding dipeptide moiety.

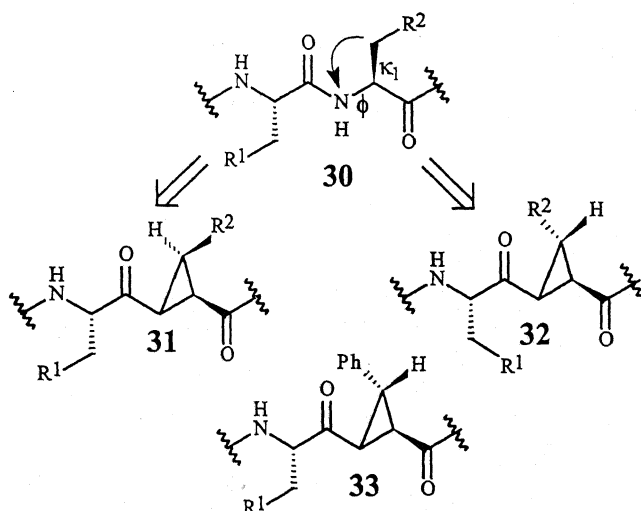


Figure-17

Operationally, **31** and **32** are derived from **30** by replacing the amide nitrogen in **30** with a carbon and forming a single bond between this atom and C(β) on the amino acid side chain.³⁸¹ The peptide backbone thus gets locally rigidified in a β -strand conformation. Another factor that has been proposed to contribute to the overall rigidity induced by this surrogate is the preferred conformation, which is a consequence of steric and electronic effects about the C-C bond between the carboxyl group and the cyclopropane.³⁸² The portion of the amino acid side chain designated as R² in the cyclopropane-derived dipeptide surrogate **31** occupies approximately the same region of space relative to the backbone that it would if the corresponding amino acid residue in **30** were locked in the gauche (-) conformation in which the κ_1 -angle is about -60 deg.

It has been reasoned that, although some hydrogen bonding capability of an amide carbonyl group is maintained by the keto functions in the rigid isosteres **31** and **32**, deletion of a backbone N-H in these surrogates eliminates one possible intermolecular hydrogen bond to a receptor or enzyme. However, the actual importance of losing this hydrogen bond is difficult to assess *a priori* since other peptide replacements that lack hydrogen bonding ability (e.g., *trans* double bonds) are known to be efficacious in certain cases. The entropic advantage that arises from restricting the rotor (0.8-1.2 kcal/rotor) in the constrained pseudopeptide could approximately compensate the energetic cost of losing one intermolecular hydrogen bond, (0.5-1.8 kcal/H-bond) provided the receptor bound conformation is well-matched.³⁸³⁻³⁸⁴ It is also significant that consequent to the mutation of the dipeptide **30** into **31** or **32** amide resonance and the associated hindered rotation about the amide -C(=O)N bond is lost, so there is now a net restriction of two full rotors. Martin *et al* discovered that **34**, which bears an N-terminal amide group rather than the keto function of the parent **31**, was a subnanomolar inhibitor of renin.³⁸⁵

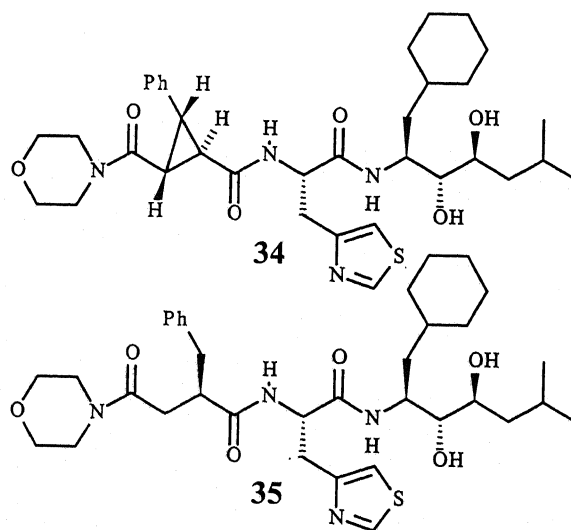


Figure-18

Since **34** was approximately equipotent with the corresponding flexible analogue **35**, it seemed probable that the two inhibitors bind to the active site of renin in closely similar conformations and that the cyclopropane ring in **34** orients the aromatic side chain at the P3 subsite in the biologically active conformation of **35**. Thus, the conformational preference of cyclopropane-containing analogues of potent acyclic inhibitors of HIV PR were studied with respect to their acyclic counterparts, to arrive at the biological conformation of the peptides.

Present study

From the above study, it is clear that constrained peptidomimics are good probes to get to the biologically active conformation of HIV PR inhibitors. It occurred to us that pseudopeptides, containing conformationally constrained phenylalanine residues as part of them, would be good leads to assist in eliciting some additional insights regarding the biologically active conformation of the inhibitors. This would also provide an insight into the scope and limitations of such pseudopeptidic replacements. In recent years, there have been a wide variety of non-peptidic amino acids reported.³⁸⁶ In general, these molecules display properly oriented side chains.³⁸⁷ However, interestingly so, almost all work has been concentrated on creating constrained analogues for proline and other P_n residues; or the $P_2 - P_n$ residues towards the N-terminal. This can be realised from a cursory look at most of the potent HIV PR inhibitors that have been shown in the preceding discussions. Apart from the work by Martin *et al.*,⁹⁶ the strategy of constraining the Phe of Phe-Pro scissile peptide bond have not been looked upon by many. We were particularly inspired by the reports of Martin *et al* that cyclopropanes may be used as structural constraints to reduce the flexibility of linear pseudopeptides and to lead to the biologically active conformations of the peptides.

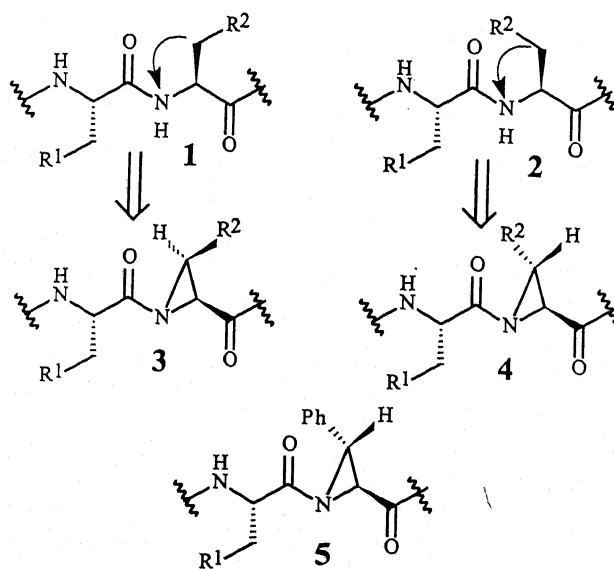


Figure-1

We wished to develop a methodology which would provide a pseudopeptidic amino acid with local constraint leading to rigidified orientation of its side chain. Consequently, we undertook efforts to develop a methodology for the synthesis of conformationally constrained mimetics for the P_1 Phe residue, which would project the amino acid side

chains in specific orientations (Figure-1). Contrary to Martin *et al.*'s design, we reasoned that retaining the nitrogen of the amide **1**, instead of replacing it with a carbon, would lead to 2-alkyl-3-amido-aziridines, which will help in an additional way. The peptide nature of the amide bond would still be retained, which might help in better recognition of the ligand by the enzyme. Introduction of an aziridine alpha to the scissile peptide bond, rather than one or more residues away from the scissile site, would render the enzyme (HIV PR) a

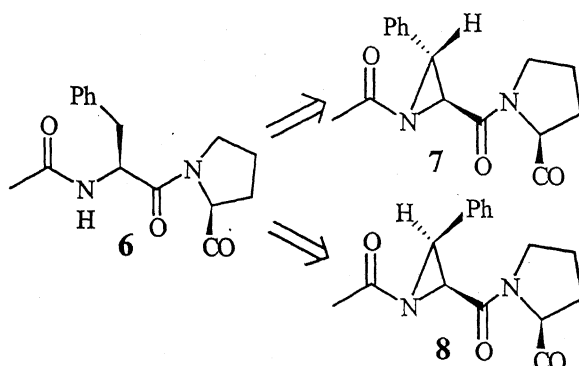
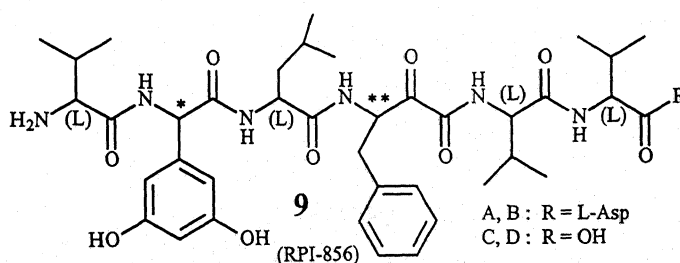
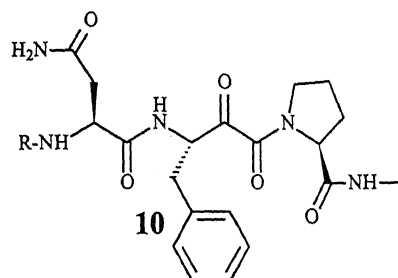


Figure-2 Mimicry of the Phe-Pro scissile bond

competitive functional group (aziridine) for binding through the aspartate groups, at its active site. Since HIV PR is uniquely specific towards cleaving the Phe-Pro amide bond, introduction of *aziridine* moiety at this bioactive Phe-Pro peptide bond would increase the chances of its better recognition by the protease. In any case, it would be interesting to study the effect of such a constraint on the selectivity of the protease, for the inhibitor. Such a strategy could lead to greater or lesser binding than the native substrate. Either case, would lead us closer to the biologically active conformation of the protein. Thus, these replacements can be incorporated at the scissile bond, in the form of Phe-Pro dipeptide isosteres.



Many potent inhibitors of HIV PR, incorporate several isosteres at the scissile Phe-Pro peptide bond site. The reports of Phe-Pro and Phe-Val pseudopeptides, containing α -keto amide isosteres (Figure-3) is illustrative of this.^{105,16}



α -Keto Amide Containing F-P & F-V Peptidomim
Figure-3

The design of Kynostatins (Kiso *et al*)²³⁹ containing the unnatural statine analogue allophenylnorstatin, incorporating the HMC isostere, is another classic example. The HMC isostere in allophenylnorstatine of the kynostatins, (for e.g., KNI 272, KNI 102, whose enzyme bound X-ray crystal structures are known)^{239,241} were designed with the premise that the Phe-Pro pseudopeptide gets recognised by the protease and the hydroxymethylenecarbonyl isostere binds to the enzyme, through the hydroxy group.

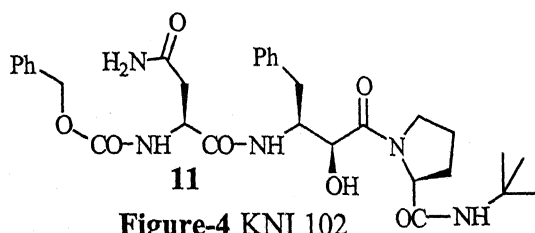


Figure-4 KNI 102

Extending this strategy of design into our aziridine containing systems, we visualized that the presence of a competitive, reactive functional group (aziridine) for acid-base catalysed hydrolytic attack by the protease active-site aspartyl groups could be a good replacement for the potent HMC or α -keto amide isosteres. Substrate mimicry, by the introduction of Phe-Pro peptidomimic, could assist in the better recognition of the pseudopeptide chain at the protease active site.

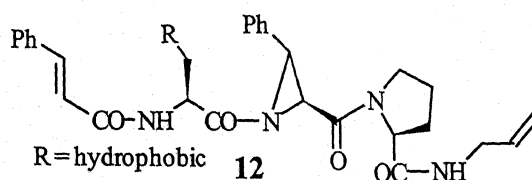
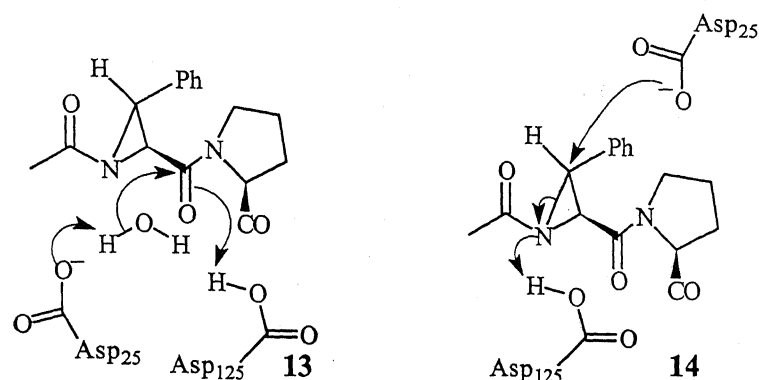


Figure-5

Moreover, the binding conformation of Phe might be as that of the aziridine, which might encourage binding with the protease. Thus, once recognised at the active site, the aspartates of HIV PR would be faced with two active functional groups for interaction, namely the Phe- ϕ -Pro carbonyl and the aziridine. Whereas the carbonyl hydrolysis needs

to be assisted by the presence of structural water; aziridine (which is highly reactive to mild acids) would undergo protonation by one of the aspartates and subsequent nucleophilic ring opening by the other at the active site, leading to irreversible binding with the protease (an analogy can be drawn from oxiranes which are known to function similarly as irreversible inhibitors).



Schematic two-dimensional representation of amide hydrolysis in the presence of Structural water; and aspartate alkylation by aziridine ring opening-14

Figure-6 Aziridine as a "Competitive Binding Element"

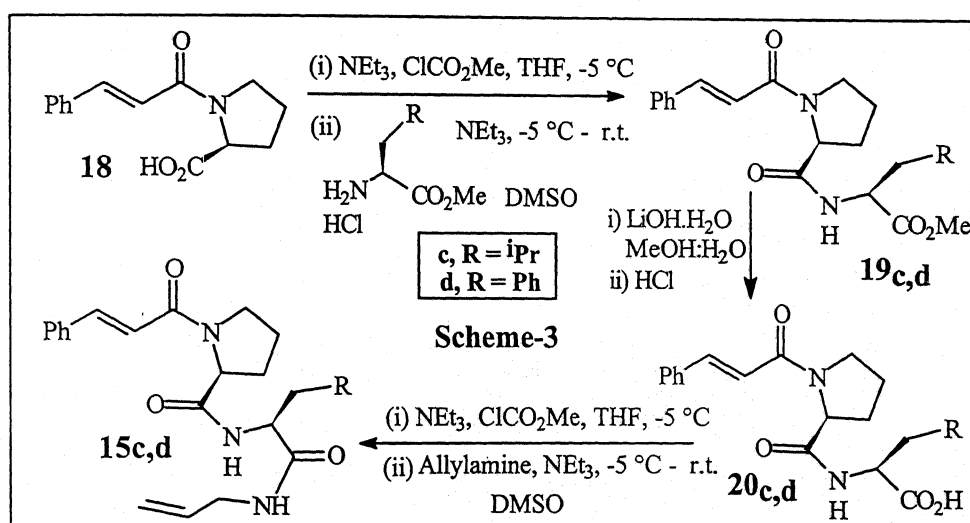
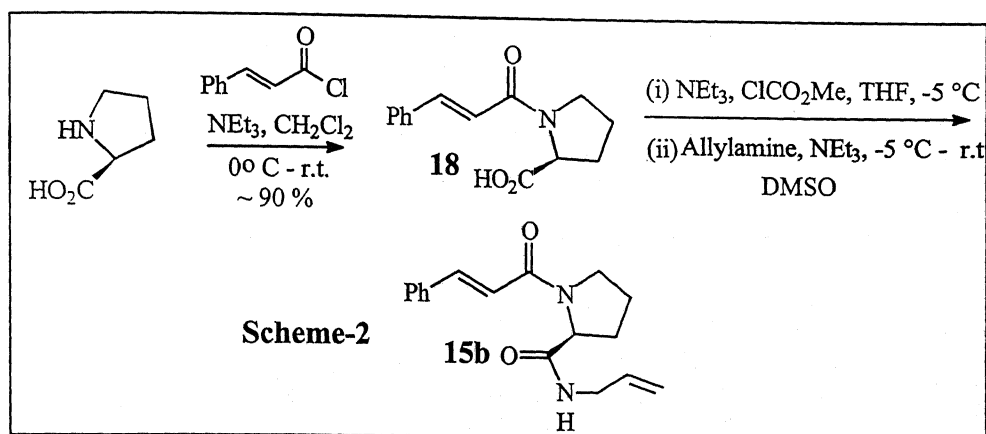
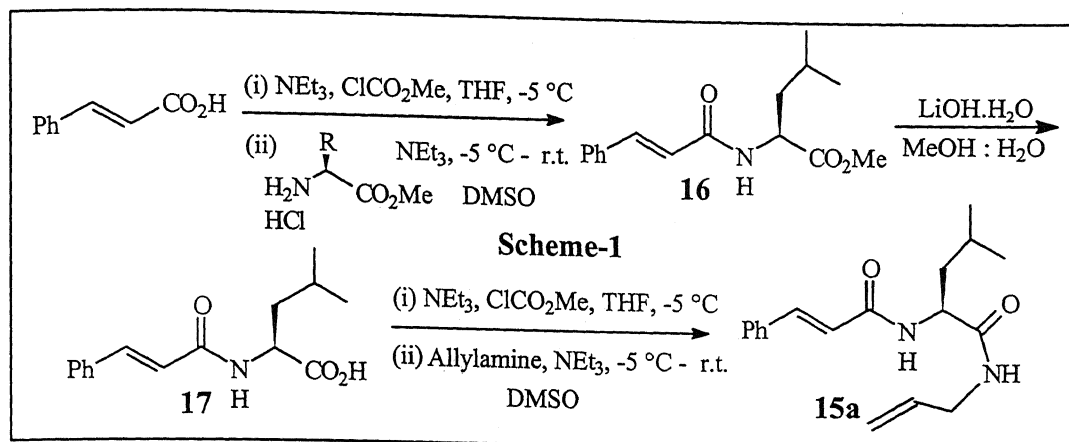
Presence of a carbocation stabilising benzyl moiety on protonation and opening of the aziridine, we reasoned, would add to assist in the competitive, irreversible binding of the ligand. Alkylation of the aspartates thus, could lead to "destruction" of the HIV PR activity. Selective recognition of any of the diastereomers of the aziridine, could lead us closer to determination of the biologically active conformation of the P₁ Phe residue at the scissile site.

The abundant presence of turns and helices at the active site of protease enzymes is well recorded. Hence, several of the conformationally restricted replacements of peptide secondary structure reported to date imitate turns or helices.⁹⁶ The presence of two constrained amino acids alpha to each other (namely, proline and constrained phenylalanine) could render, either a β -strand conformation as noted by Martin *et al.* (on introduction of cyclopropane as conformational constrain) or a turn conformation, to the peptide backbone. Hence, rendering the peptide an additional element of recognition for the protease active site. On the basis of the above considerations, we reasoned that conformationally constrained Phenylalanine (CCP)-Pro containing peptides could be excellent irreversible competitive inhibitors of aspartyl proteases and could be good probes to lead us to the biologically-active conformation of inhibitors.

The synthetic foundation on which such a design was based was the excellent yields, observed for the epoxidation of N-cinnamoyl peptides to obtain the corresponding epoxide containing peptides, in the presence of PASCOS catalyst. Ring opening of epoxides with sodium azide is known to follow exclusively *anti* stereochemistry to lead to the azido alcohols. Conversion of these azido alcohols to aziridines³⁸⁸ have been reported to be achieved by refluxing with triphenylphosphine, in good yields. Hence, the epoxides of N-cinnamoyl-peptides can be converted to the corresponding aziridine containing peptides, as conformationally-constrained-phenylalanine (CCP) containing peptidomimics. Acylation of these aziridines with N-protected amino acids, would lead us to ligands with amino acids of our choice at the Pn & Pn' sites. However, crippled by the immediate non-availability of high throughput screening facilities, we set to develop a general methodology for the facile introduction of CCP-Pro peptidomimic at any required junction of a peptide framework.

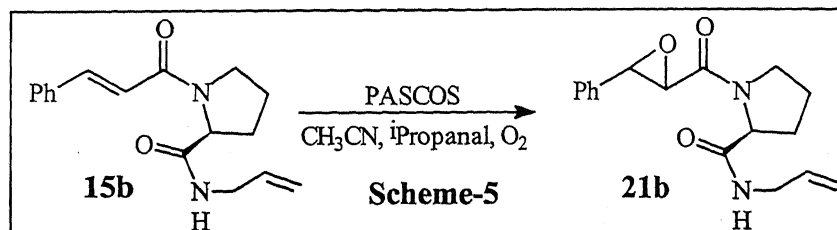
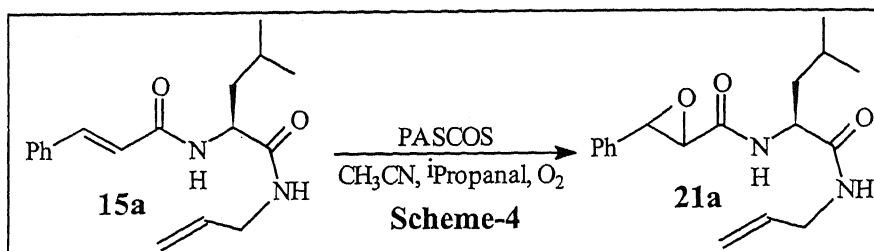
The HIV PR active site is characterized by the presence of large S₁, S₂ hydrophobic pockets. Hence, introduction of P₂ site aminoacid residues containing hydrophobic side chains (e.g. Leu, Phe) would help to vary the binding and recognition of the ligand with the active site. We wished to introduce novel N- and C-terminal protecting groups in the form of cinnamoyl and allylamide motifs respectively. The cinnamoyl protecting group, as shown earlier, could be a "*pro-functional hanger*" for further introduction of structural diversities in the peptide. Allyl amide was used, owing to our observation that presence of a C-terminal amide, rather than an ester, rendered higher stereoselectivity during the epoxidation of the cinnamoyl double bond, in the presence of PASCOS (polyaniline supported cobalt salen). Added to this, ligands whose C-termini were protected as amides, are known to be better inhibitors than the corresponding esters. Thus, with the above premise we set to synthesize the aziridine containing peptides, through our epoxidation protocol in the presence of PASCOS.

The N-cinnamoyl protected peptide derivatives **15a-d**, namely allyl-N-cinnamoyl-L-proline-amide, allyl-N-cinnamoyl-L-leucine-amide, allyl-N-cinnamoyl-L-proline-L-leucine-amide and allyl-N-cinnamoyl-L-proline-L-phenylalanine-amide were synthesised in good yields from their corresponding carboxylic acids by coupling with allyl amine using the mixed anhydride coupling procedure, with isobutyl chloroformate as the carboxyl activating group.

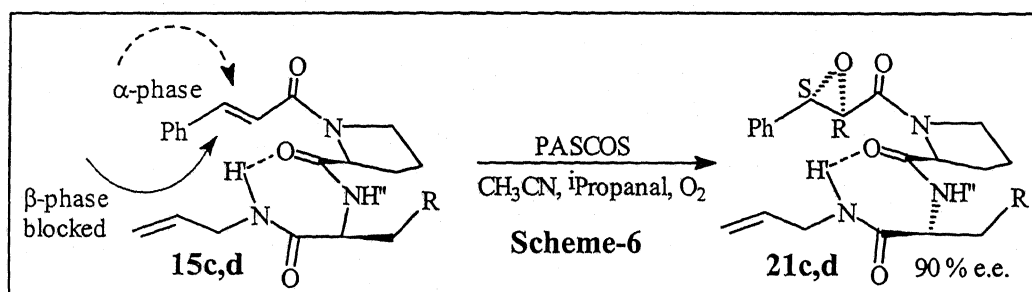


These N-cinnamoyl peptides **15a-d** were subjected to epoxidation conditions, in the presence of PASCOS and 2-methylpropanal, in aerobic conditions. Typically, the olefinic peptide was taken in acetonitrile and addition of 2-methylpropanal (2 equivalents), was followed by the addition of PASCOS catalyst after which, the reaction mixture was stirred for 12 h in oxygen atmosphere. Additional lots of PASCOS catalyst and 2-methylpropanal were added, after stirring for 12 h and the reaction mixture was stirred

till completion consumption of olefin (~20-24 h), in the case of **15a,b**; however, additional amounts of catalyst and aldehyde were needed in three lots, after 12 and 24 h in the case of **15c** & **15d**, for near-completion of the reaction (~36-40h).

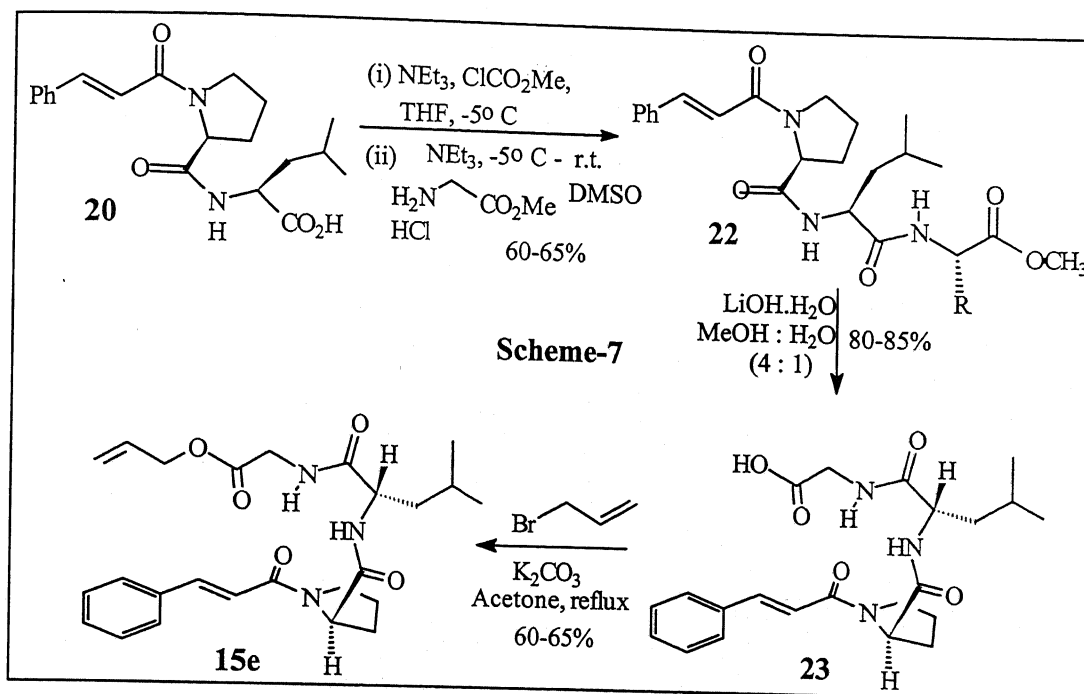


Removal of solvent, taking the resulting residue in EtOAc and washing with sodium bicarbonate and water, yielded the corresponding epoxides in moderate to good yields. While **21a,b** were formed in excellent yields as usually observed; unlike in thus far seen cases, the reaction period and the quantity of aldehyde & catalyst required for the epoxidation of **15c,d** were quite high.

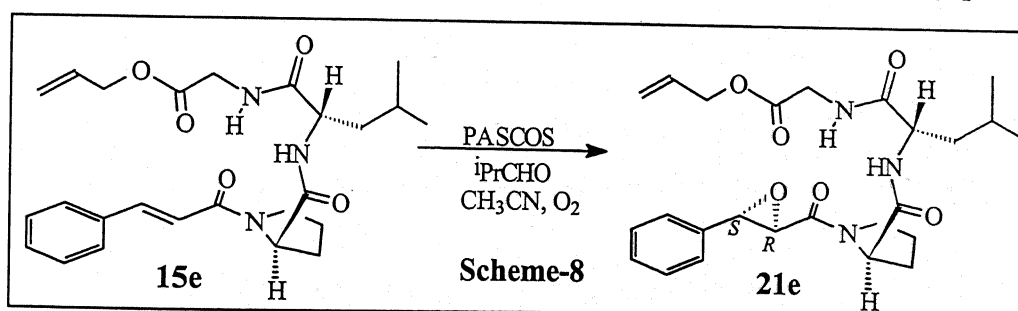


The yields of **21c,d** were also quite lower than in thus far observed cases. While conversion was never complete, the reaction mixture contained highly polar (soluble only in methanol/DMSO) oligomeric material, which were not characterised. We reasoned that isobutyric acid that is generated in the reaction mixture could be the culprit, added to the long reaction times. The allyl amide might itself be undergoing oxidation, hence leading to further complications in the reaction. Further, on extending the epoxidation protocol to the allyl-N-cinnamoyl-tripeptide ester **15e**, we found that the required epoxide was formed in very poor yields (~5-10 %) after long reaction times (~60 h) along with a score of side products. **24** was synthesized from N-cinnamoyl-proline-leucine, as

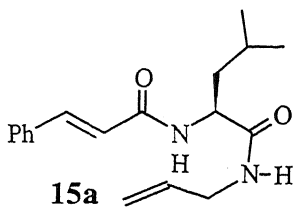
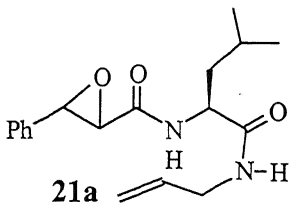
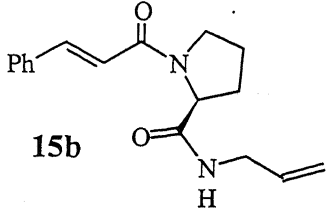
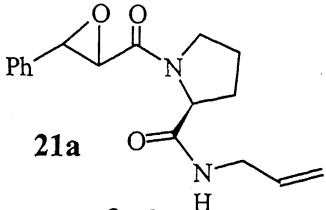
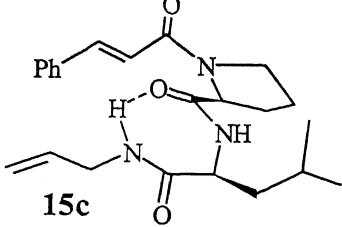
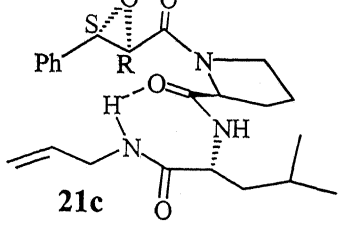
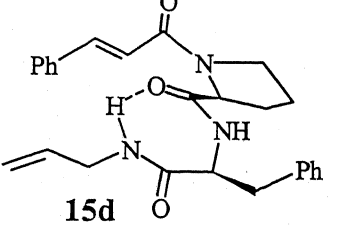
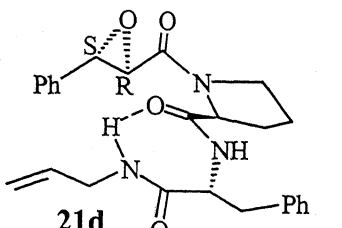
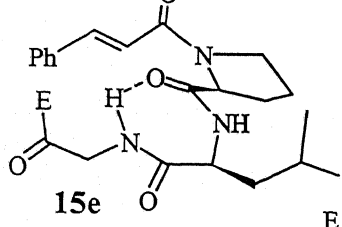
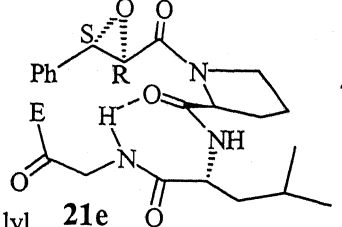
shown in Scheme-7. Glycine was chosen as the third amino acid for its simplicity. The poor yields of epoxide and the formation of number of other oxidised side products, meant that the presence of a third amide functionality had a telling effect on the epoxidation. We reasoned that buffering the reaction might help in reducing the reaction period, which could reduce side reactions.



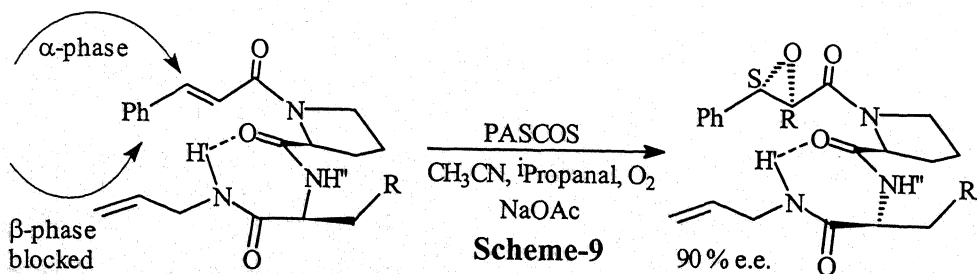
PASCOS Catalysed Facial Selective epoxidation of N-Cinnamoyl-tripeptide



PASCOS Catalysed epoxidation of allyl-N-cinnamoyl-peptido amides

| Substrate | Epoxide | Yield, (3S2R : 3R2S) ^a |
|---------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------|--------------------------------------|
|  15a |  21a | 92 % ~1:1 |
|  15b |  21a | 89 % 59:41 |
|  15c |  21c | 62 % 9.5:0.5 |
|  15d |  21d | 64 % 9.5:0.5 |
|  15e |  21e | ~10 % >10:1 |

E = Oallyl

a - Determined from ¹H NMR**Table-1****Substrate Directed Turn Induced Facial Selective Epoxidation**

Hence, the oxirane containing peptides **21b,c,d,f** & **21g** were obtained by the following epoxidation conditions. To a solution of the oxirane containing peptide in acetonitrile was added 2-methylpropanal (2 equivalents) and PASCOS catalyst, followed by sodium acetate (10 equivalents) and the reaction mixture was stirred for 12 h. While **15b** underwent complete conversion in that time to the corresponding epoxide **21b** in excellent yields; **15c,d** needed another lot of the aldehyde and the catalyst, to result in the

Comparison Between Buffered and Unbuffered Epoxidation Reaction in The Presence of PASCOS

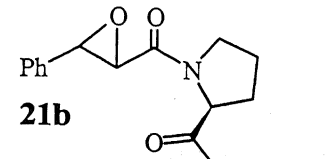
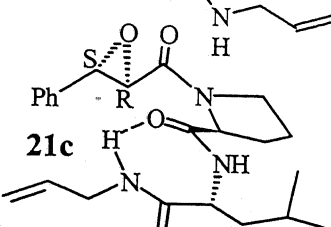
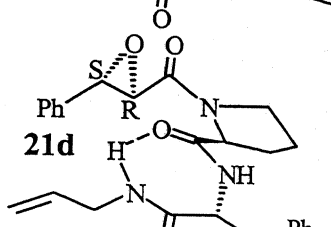
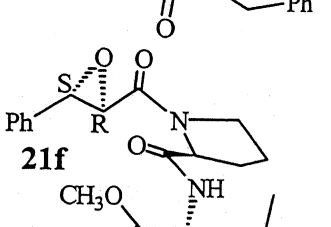
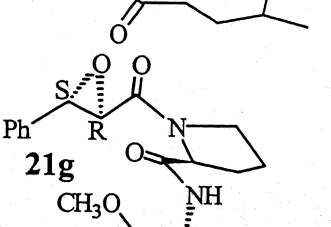
| Epoxide | No Sodium Acetate | | In Presence of Sodium Acetate | |
|---------------------------------------------------------------------------------------------------|-------------------|------|-------------------------------|------|
| | Yield | Time | Yield | Time |
|  21b | 89 % | 22 h | 92 % | 15 h |
|  21c | 62 % | 36 h | 89 % | 20 h |
|  21d | 64 % | 33 h | 93 % | 17 h |
|  21f | 86 % | 21 h | 90 % | 14 h |
|  21g | 90 % | 24 h | 89 % | 14 h |

Table-2

corresponding epoxides **21c,d** respectively, in excellent conversion and yields, in 20-22 h. However, there was no significant change in the rotation and hence the facial

selectivity, on buffering the reaction. Similar was the observation for the epoxidation of the N-cinnamoyl-dipeptide-methyl esters, **21f,g**. While yields were similar as in the case of unbuffered reaction, the reaction time and the amount of catalyst required, went down quite significantly (Table-2). Thus, buffering the epoxidation reactions of N-cinnamoyl peptides with sodium acetate, resulted in faster reactions leading to excellent yields of the corresponding epoxides.

Facial Selective Epoxidation

The epoxidation of allyl-N-cinnamoyl-peptide amides, in general exhibited excellent facial selectivities during PASCOS catalysed epoxidation. We were thrilled to observe excellent diastereoselectivity during the epoxidation, especially of **15c,d** to the corresponding epoxides **21c,d**, in good yields. The NMR of the epoxides showed the presence of two pairs of epoxide methine doublets (9.5 : 0.5), as observed in the case of the corresponding methyl and allyl esters. From the negative sign of rotation of the epoxides and NMR correlation of the epoxy methine chemical shifts, we assigned a stereochemistry of (3*S*, 2*R*) to the predominant, epoxide diastereomer. ¹H NMR studies on the olefinic peptides **15c,d**, showed the presence of low field triplet (7.86, t, *J* = 8.8 Hz) indicating the presence of hydrogen bond in the molecule involving the allyl amide -NH. We reasoned that this could have led to a structural pre-organisation in the olefinic peptide, resulting in facial bias of the double bond for epoxidation. However,

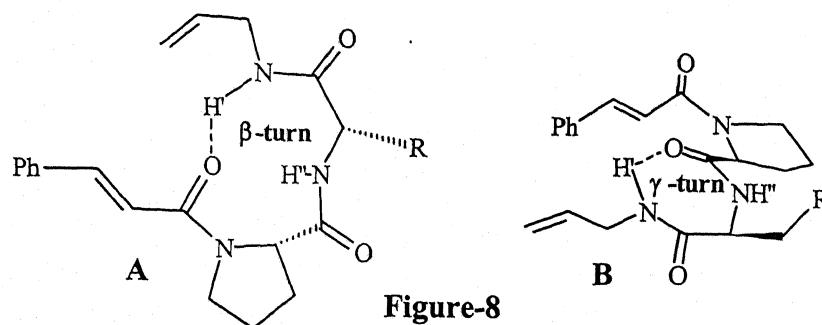
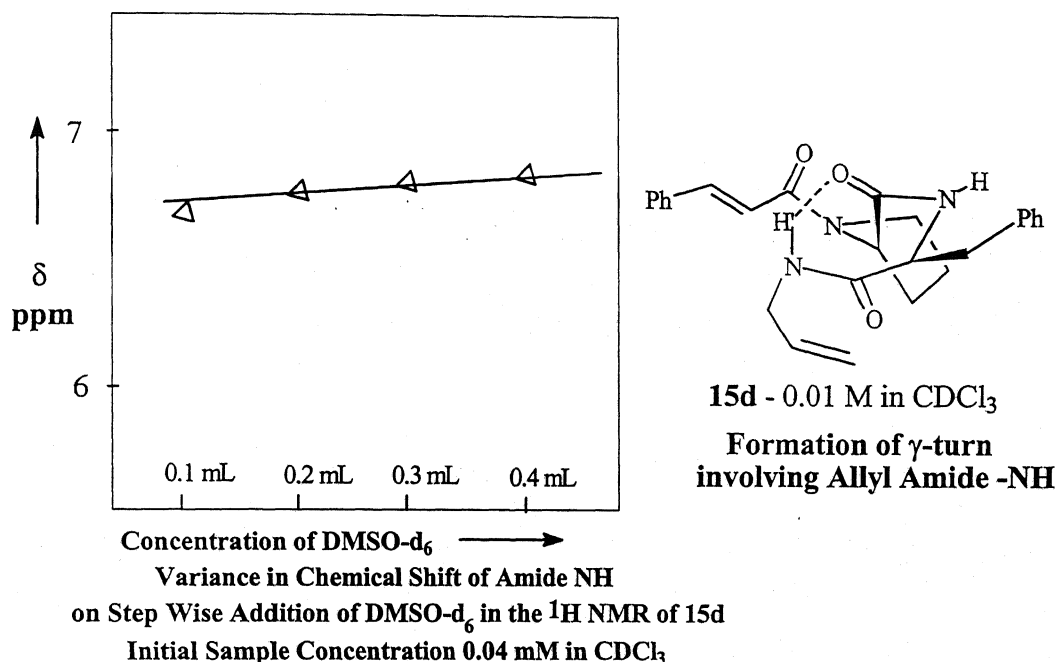
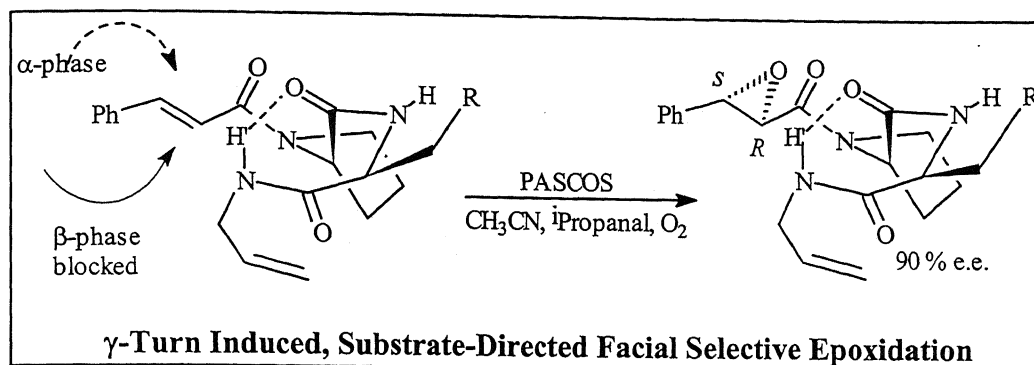


Figure-8
Three possible Hydrogen Bonding Interactions Induced by Turn

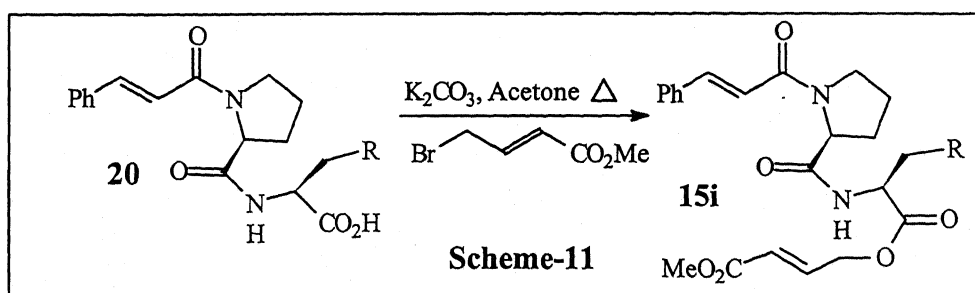
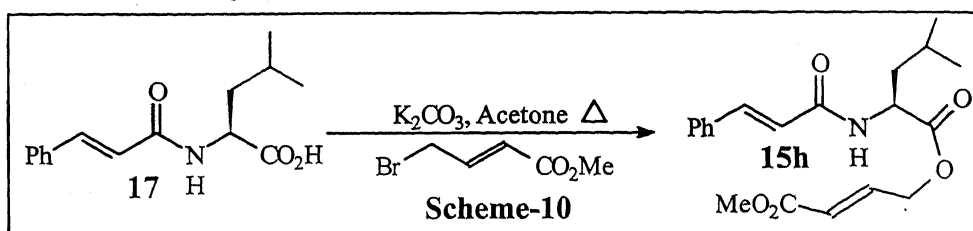
there are two possible hydrogen bonding interactions involving the allyl amide -NH. One, leading to a β -turn induced conformer; and the other leading to a *gamma* turn induced conformer.



It is known that chemical shifts of intramolecularly H-bonded protons do not vary appreciably in the ¹H NMR of such systems, on addition of high concentrations of polar solvents like DMSO-d₆ to the solution. ¹H NMR studies of the N-cinnamoyl-peptide **15d** by gradual addition of DMSO-d₆ to increase the polarity of the solvent showed that among the two amide NH hydrogens, there was negligible shift in the chemical shift of H', whereas H'' shifted down field on addition of DMSO-d₆ to solution. Thus, H' was involved in hydrogen bonding. An examination of the energy minimised molecular models of the olefins on the Hyperchem energy minimisation software, using MM⁺, indicated the presence of a *gamma* turn induced hydrogen bonding, formed between the NH of the allylamide and the carbonyl of proline. This *gamma* turn motif, clouds the β-phase of the cinnamoyl double bond, creating a huge facial bias for epoxidation. The cinnamoyl moiety preferred a *cisoid* geometry. The proline residue was more convenient

in adopting a *cis* conformation rather than *trans*. All these indicated towards the observed stereoselectivity of epoxidation. From NMR, chemical correlation and molecular modelling studies, we propose that the observed stereoselectivity in epoxidation could be due to the substrate-directed facial bias of the cinnamoyl double bond, caused by the clouding of the *gamma* turn motif on one of its faces. That this could be the solution conformation, is also supported by the lesser diastereoselectivity observed, in the epoxidation of the corresponding allyl, methyl, and crotonate esters, which lack the possibility of such a *gamma* turn induced secondary structure on their side chain.

Synthesis of C-Terminal Extended Peptides



PASCOS Catalysed Epoxidation of C-Terminal Extended Peptides

| N-Cinnamoyl peptide | Epoxide | Yield [α] _D ^{CH₂Cl₂} | (3S,2R : 3R,2S) ^a |
|---------------------|----------------|----------------------------------------------------------------------------|------------------------------|
| 15h | 21h | 90 % - 8 | ~ 5:4.5 |
| 15i | 21i | 90 % - 108 | >9:1 |

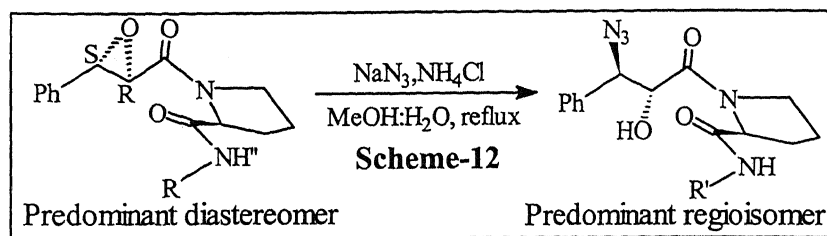
E = CO₂Me

We had observed earlier that replacing the methyl ester with allyl ester resulted in better stereoselectivity (^1H NMR), during epoxidation of N-cinnamoyl-peptides. In order to ascertain whether stereoselectivity of CPX systems (see Part-A-section-II) could indeed be due to selective clouding of one of the faces of the olefin, we synthesised the peptides **15h,i** with extended C-terminal esters. γ -bromo-methylcrotonate was chosen for C-terminal extension. γ -Bromo crotonate was reacted with N-cinnamoyl-leucine (**17**) and N-cinnamoyl-proline-leucine (**20**) in the presence of potassium carbonate in acetone, to give the corresponding O-alkylated peptides **15h,i** in good yields (Scheme-10,11). Subjection of the crotonate derived peptides **15h,i** to epoxidation conditions in the presence of PASCOS and 2-methylpropanal under oxygen atmosphere, yielded the corresponding epoxides **21h,i** in excellent yields. As observed earlier, the epoxidation was chemospecific towards the cinnamoyl double bond and in the case of **15i**, the stereoselectivity of epoxidation was higher (8.3:1.7) than that observed for the case of the corresponding methyl or the allyl esters. However, there was little increase in stereoselectivity in the case of the formation of **21h**, than in the case of the corresponding methyl and allyl esters.

Thus, PASCOS catalysed epoxidation of N-cinnamoyl-pyrrolidine containing peptides yield epoxides in a highly stereoselective manner, the selectivity increasing with increasing chain length. In the case of N-cinnamoyl-tripeptides, the facial selectivity is found to be excellent (*e.e.* ~90%). Presence of a γ -turn induced secondary structure that induces a facial bias for one of the faces of the cinnamoyl double bond, seems to be the reason for the same. That this γ -turn induced H-bonding persisted even after epoxidation, was seen by the insignificant variation of chemical shift in the ^1H NMR for the allyl amide triplet on subjection to DMSO- d_6 addition studies (Figure-11).

Conformationally Constrained Phenylalanine (CCP) Containing Peptides

Aziridination, via opening of the epoxides with sodium azide and its subsequent closure in the presence of triphenylphosphine, is known to take an $\text{S}_{\text{N}}2$ pathway and result in inversion of stereochemistry. Extending this methodology of aziridination into our systems would result in the formation of aziridine containing peptides, from the corresponding oxirane containing peptides.

**Table-4** **Synthesis of Azido Alcohols**

| Epoxide | Azido Alcohol ^b | Yield | regiomers ^c ($\alpha : \beta$) ^a |
|-------------------|----------------------------|-------|-------------------------------------------------------------|
| <p>21a</p> | <p>25a</p> | 86 % | 1 : 1 |
| <p>21b</p> | <p>25b</p> | 93 % | 1 : 1 |
| <p>21c</p> | <p>25c</p> | 79 % | 1 : 2.3 |
| <p>21d</p> | <p>25d</p> | 75 % | 1 : 3 |
| <p>21e</p> | <p>25e</p> | 83 % | 1 : 2 |

a = With Respect to the Cinnamoyl Carbonyl; b = Major Regio Isomer shown; c = Regiomers is Abbreviated for Regio-Isomer

As discussed earlier, these can be considered as good conformationally constrained mimics of phenylalanine. Thus, the oxirane containing peptides **21a-e**, were reacted with sodium azide and ammonium chloride to get to the corresponding azido alcohols. As for

the initial hick-ups, the reaction did not proceed well in DMF or methanol in dry and ambient conditions.

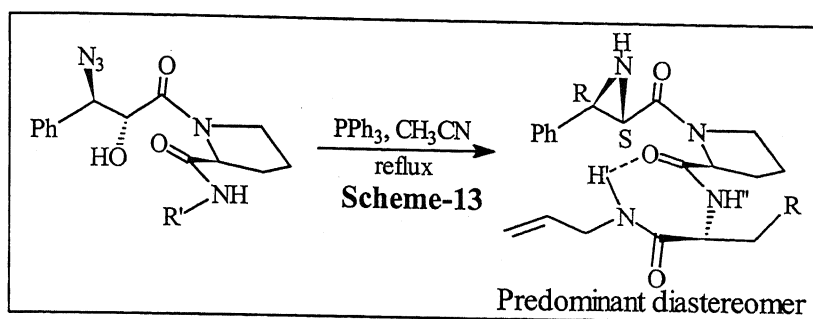


Table-5 Synthesis of CCP Containing Peptides

| Azido Alcohol | CCP Containing ^a Peptide | Yield % | $[\alpha]_D^{CH_2Cl_2}$ |
|-------------------|-------------------------------------|---------|-------------------------|
| <p>25a</p> | <p>26a</p> | 8 % | -0.5 |
| <p>25b</p> | <p>26b</p> | 54 % | - b |
| <p>25c</p> | <p>26c</p> | 68 % | -94 |
| <p>25d</p> | <p>26d</p> | 65 % | -79 |
| <p>25e</p> | <p>26e</p> | 70 % | -139.7 |

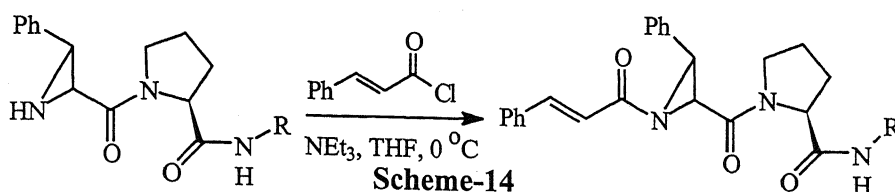
a - Predominant diastereomer is shown; b - did not give a constant value of rotation

However, on refluxing the epoxy peptides in methanol:water solution, with sodium azide and ammonium chloride filtration, concentration & washing with water, and purification by column chromatography (EtOAc:hexane), yielded the azido alcohol as a mixture of regio isomers of which the benzyl azide products were found to be the predominant regioisomer (^1H NMR) in each of the cases.

To a solution of these azido alcohols **25a-e** in dry acetonitrile was added triphenylphosphine in room temperature (in portions) and the reaction vessel was set to reflux. After completion of reaction, the solvent was removed and the contents isolated by subjection to flash column chromatography to yield the required aziridine containing peptides **26b-e** in good yields. However, in the case of **25a**, the corresponding product **26a** was formed in poor yields.

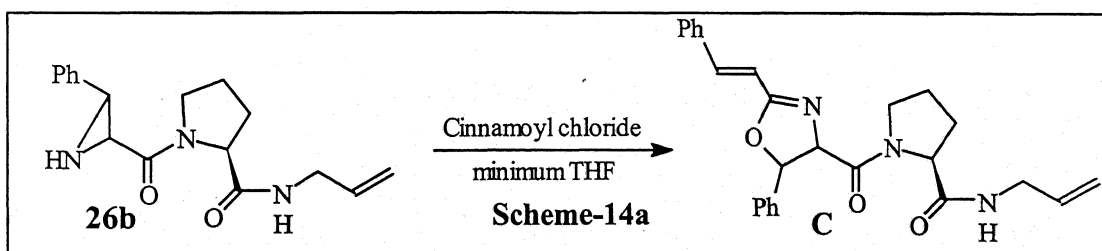
Subjecting **26c** to DMSO- d_6 solvent addition studies (Figure-12) revealed the huge shift of the allyl amide triplet in its ^1H NMR. This shift suggested that incorporation of aziridine as part of the peptide, probably disrupts the γ -turn induced H-bonding that persisted in the olefins (**15c,d**) and the corresponding epoxide containing peptides (**15c,d**). It is thus probable that aziridine, as part of the peptide system, could impart rigidified linearity to the peptide. The energy minimized conformational studies of the aziridine containing peptide (**26c**) also showed that the peptide adopted a linear minimum energy conformation.

These aziridines were acylated with cinnamoyl chloride in THF. Typically, to a solution of the aziridine in THF was added triethylamine and the reaction mixture was cooled to 0 °C. To the stirring solution was added a dilute solution of cinnamoyl chloride in THF and the mixture stirred till completion of reaction. Removal of solvent, washing with bicarbonate & water, drying, concentration and purification by column chromatography yielded the corresponding cinnamoylated aziridine containing peptides, in good yields. Reaction of cinnamoyl chloride, however, in minimum amount of solvent (THF) led to the formation of five membered amino ketal ring system as the major product, as exemplified by the formation of **C** from **26b** (Scheme-14a). Thus, the CCP-Pro containing peptides **26b-e** were synthesised from the corresponding aziridine containing peptides **25b-e**. These CCP-Pro containing peptides can thus have a diversity at the P_2' site and the C-terminal protecting groups.

**Table-6**

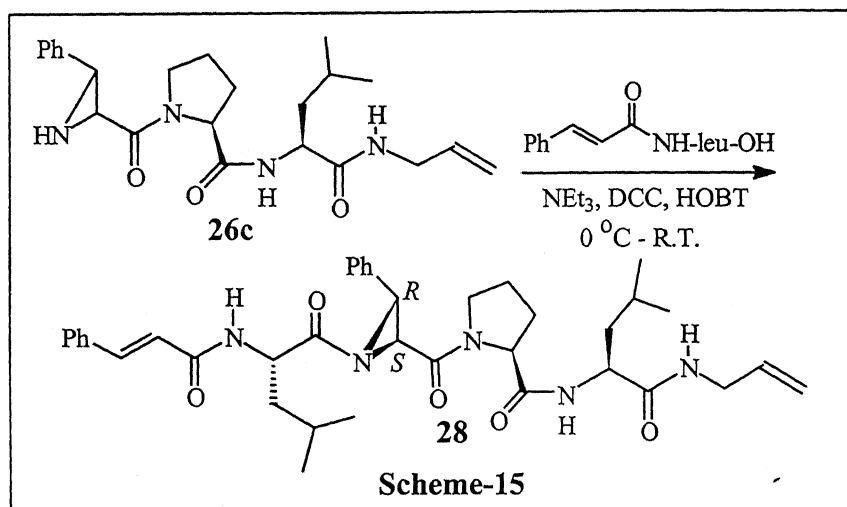
Synthesis of N-Cinnamoyl-CCP-Pro Containing Peptides With Diversity At P2', P3' S

| N-Truncated CCP Peptide | N-Cinnamoyl CCP Peptide ^a | Yield % | $[\alpha]_D^{CH_2Cl_2}$ |
|-------------------------|--------------------------------------|---------|-------------------------|
| <p>26b</p> | <p>27b</p> | 72 % | + 32 |
| <p>26e</p> | <p>27e</p> | 56 % | + 12 |
| <p>26c</p> | <p>27c</p> | 75 % | + 76 |
| <p>26d</p> | <p>27d</p> | 75 % | + 57 |
| M = -HN-Allyl | | | |

^a - predominant diastereomer is shown

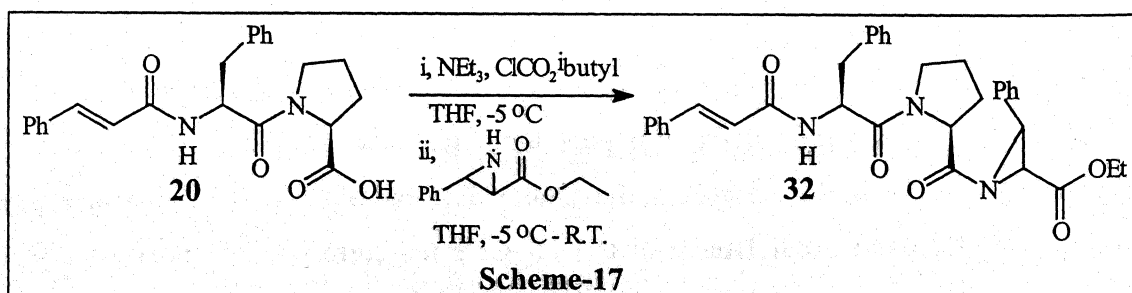
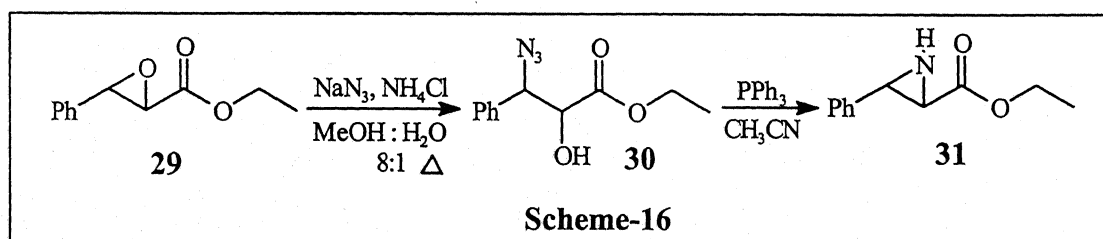
Diversities can also be introduced at the P2 site of these peptides containing the conformationally constrained Phe-Pro peptidomimic. Thus, coupling of N-cinnamoyl-leucine **17** with **26c** in the presence of dicyclohexylcarbodiimide (DCC), 1-hydroxybenzotriazole (HOBT) and triethylamine in CH_2Cl_2 gave the corresponding pentapeptide **28**

containing the Phe-Pro peptidomimic. Thus, diversities could also be introduced at the P2 site of these CCP-Pro containing peptides.



Conformationally Constrained Phe at any part of the peptide

The CCP amino acid residue can be incorporated at any desired site of the ligand (peptide). We synthesised a Pro-CCP containing peptide. PASCOS is known to catalyse the epoxidation of ethyl cinnamate to ethyl phenylglycidate in good yields, in the presence of 2-methylpropanal and oxygen atmosphere in acetonitrile. Opening of commercially available ethyl-3-phenyl-glycidate 29 with sodium azide in ambient conditions in the presence of ammonium chloride yielded the corresponding azido alcohol 30, which was refluxed in acetonitrile with triphenylphosphine to yield the corresponding aziridine containing derivative 31 in good yields.



Coupling of **31** with N-cinnamoyl-phenylalanine-proline using the mixed anhydride coupling procedure, with isobutylchloroformate as the carboxyl-activating group yielded the corresponding pseudo tetrapeptide **32**. This is isostructural to methyl-N-cinnamoyl-phenylalanine-proline-phenylalaninate **33**, which was synthesised by the coupling of appropriate amino acid residues in the required sequence using the mixed anhydride coupling procedure (Scheme-18). **27d** and **32** are isostructural to **33** (Figure-10).

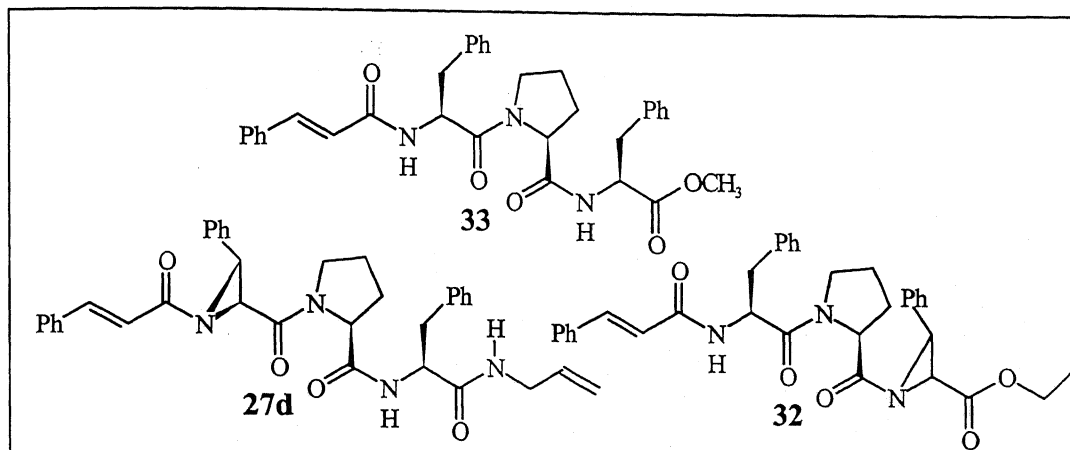
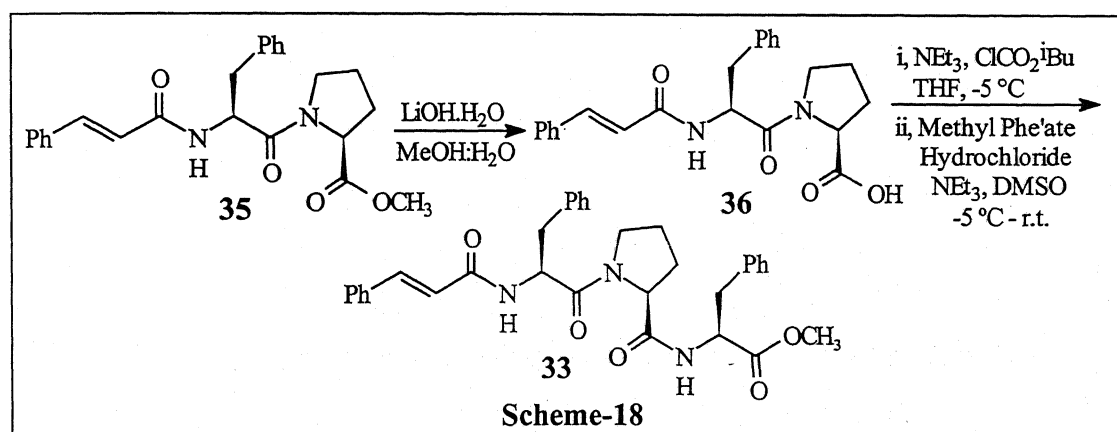


Figure-13

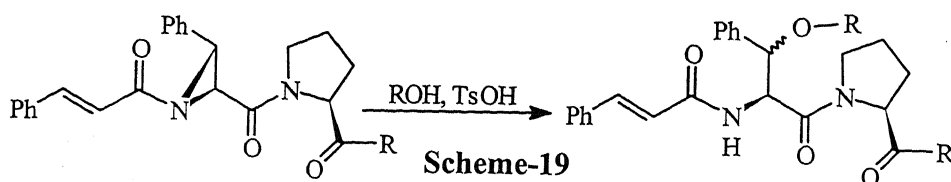


Scheme-18

The CCP-Pro peptidomimic containing peptide **27c** was subjected to acid catalysed ring opening, in the presence of catalytic amount of *p*-toluenesulphonic acid with methanol / water / allyl alcohol, to yield the corresponding β -substituted phenylalanine-derivative containing oligopeptides **34a-c** in good yields.

The facile opening of the aziridine containing peptide, suggests that these pseudopeptides might be good competitive irreversible inhibitors of HIV PR, in the presence of the rtyl groups. These oligopeptides **34a-c** could also be crucial in leading us to conformation of the inhibitor ligands at the HIV PR active site; since,

presence of C_β -substituents on the phenylalanine would impose conformational restrictions on the phenylalanine residue.



| β -Substituted Phe Containing Peptide | Yield | $[\alpha]_D^{CH_2Cl_2}$ |
|---------------------------------------------|-------|-------------------------|
| <p>34a</p> | 71 % | - 11 |
| <p>34b</p> | 66 % | - 8 |
| <p>34c</p> | 60 % | -a |

a - did not give a constant value of rotation

Thus, we have designed and synthesised aziridine-containing peptides, incorporating a conformationally constrained 2-phenyl-acylaziridine as potential competitive Phe-Pro peptidomimic for irreversible binding at the HIV PR active site. These constrained analogues could assist in imparting a β -strand conformation, which is a common recognition element at the active site of HIV PR, to the oligopeptide backbone. The C_β -substituted phenylalanine-derivate containing oligopeptides derived from the aziridine analogues, could also act as conformationally constrained phenylalanine analogues. We have discovered that N-cinnamoyl-pyrrolidine containing peptides impart facial selectivity to the cinnamoyl double bond, during epoxidation. Presence of a third peptide bond containing an amide NH, leads to greater stereoselectivity during epoxidation due to the presence of a hydrogen-bonded motif - possibly a *gamma* turn formed by the hydrogen bonding between the -NH hydrogen of the third amide and the proline

carbonyl. NMR, chemical correlation and molecular modeling studies seem to supplant such reasoning.

Experimental Section

Materials and Methods

Acetonitrile, ethyl acetate, hexane, THF and all other solvents were purified by standard procedures. All the amino acids were bought from SpectroChem India Limited and used as such. Cinnamic acid, methylchloroformate, sodium azide, isobutylchloroformate, triethyl amine, triphenylphosphine, ammonium chloride, 2-methylpropanal (isobutyraldehyde) were all procured commercially and were purified before use. Polyaniline supported Co(II) Salen was prepared according to procedure developed in our lab.¹⁸² Column chromatography was performed on ACME silica-gel eluant. TLC was performed on ACME silica-gel-G coated glass plates and were visualized using UV lamp. ¹H NMR spectra were recorded using Jeol PMX-60 system, Bruker WP-80, Jeol 300 FTNMR or JNMLA400 FTNMR machines in CCl₄/CDCl₃. Chemical shifts are given relative to TMS in ppm(δ). Multiplicity is indicated using the following abbreviations: s(singlet), bs(broad singlet), d(doublet), dd(doublet of a doublet), ddd(doublet of a doublet of a doublet), dt(doublet of a triplet) td(triplet of a doublet), q(quartet), dq(doublet of a quartet), tdd(triplet of a doublet of a doublet), qd(quartet of a doublet) and m(multiplet). The FAB mass spectra were recorded on JEOL SX 102/DA 6000 mass spectrometer data system using Argon (6 Kv, 10 mA) as the FAB gas. Optical rotations were measured in Autopol[®] II/ Autopol[®] III polarimeters. All the known compounds were characterized by comparing with the literature data. IR spectra were recorded on Perkin Elmer 683 spectrophotometer, using either a neat sample or a solution in CCl₄/CH₂Cl₂ and solids were examined as KBr pellets and the values are reported in ν_{\max} (cm⁻¹). HPLC analyses were done with Rainin System fitted with Dynamax[®] SD-200 pump and detected with Groton PDA solonet Diode Array Detector.

General Procedure for the Synthesis of methyl-L-amino ester hydrochloride

To an ice cold, stirring suspension of the L-amino acid (1 equivalent) in methanol (1 mL/mmol) was added thionylchloride (1.1 equivalents) drop wise from a dropping funnel. The clear solution that resulted was stirred at room temperature for 3 h. Methanol was removed in vacuo and the resulting solid was washed with dry ether. Drying under vacuo yielded the amino ester hydrochloride in nearly quantitative yields, which was used for further reaction in the next step without any further purification.

In general it was observed that coupling reactions with methyl-amino ester hydrochlorides were better yielding when these were freshly synthesised before use for subsequent synthesis, due to the hygroscopic nature of most of these salts.

General Procedure for the Synthesis of methyl-N-cinnamoyl-amino ester

Method A

To a stirring, ice cold solution of cinnamic acid (1 equivalent) and triethylamine (1 equivalent) in THF (1.5 mL/mmol) was added methylchloroformate (1 equivalent) and the mixture was stirred vigorously for 2 minutes.* After which, a solution of the amino ester hydrochloride (1.1 equivalents) in DMSO (0.5 mL/mmol) was added followed by triethylamine (2.2 equivalents) dissolved in THF (1 mL/mmol). The reaction vessel was allowed to warm to room temperature and vigorously stirred for further 3-4 h. Triethylamine hydrochloride was filtered off on a sintered funnel under suction and washed with THF. Removal of solvent from the filtrate under vacuo yielded a residue,

which was dissolved in EtOAc (~ 2mL/mmol) and washed with saturated aqueous solution of NaHCO₃, water and brine. Drying (Na₂SO₄) and evaporation of solvent under vacuo yielded the crude product which was further purified by column chromatography (EtOAc:Hexane) to yield the required product usually as good solids in good yields.

* On prolonging beyond this time, usually methyl cinnamate is formed in healthy quantities as a side product.

Method B

To a stirring ice cold solution of cinnamoyl chloride (1 equivalent) in dichloromethane (1 mL/mmol) was added the amino ester hydrochloride (1.1 equivalents) followed by a solution of triethylamine (2.2 equivalents) in dichloromethane (1 mL/mmol) drop wise through a dropping funnel. After complete addition of triethylamine, the reaction mixture was vigorously stirred for a further 5-6 h and then diluted with dichloromethane (1 mL/mmol). Work up as described in method A, with saturated aqueous solution of NaHCO₃, water and brine and purification by column chromatography yielded the N-cinnamoyl amino ester in good yields.

In general, it was observed that yields of methyl-N-cinnamoyl-amino esters were better by method A, than by method B.

General Procedure for the Synthesis of N-cinnamoyl-amino acid

Method A

To an ice cold solution of cinnamoylchloride (1 equivalent) in dichloromethane (1 mL/mmol) was added the L-amino acid (1 equivalent). To the stirring mixture a solution of triethylamine (2.2 equivalents) in dichloromethane (1 mL/mmol) was added drop wise at such a rate that the vessel temperature did not exceed 10 °C. After complete addition, the reaction mixture was warmed to room temperature and stirring was continued for 4-5 h. The solvent was evaporated in vacuo. To the resulting residue was added while stirring, aqueous solution of 1N HCl until no more acidification occurred (formation of milky white precipitate ceased). The aqueous suspension of yellowish white solid was filtered on a sintered funnel under suction and dried by spreading on cellulose filter paper for 10 h in a dessicator, to yield the N-cinnamoyl amino acid in excellent yields.

Method B

To a solution of the methyl-N-cinnamoyl amino ester (1 equivalent) in MeOH (4 mL/mmol) was added a solution of LiOH.H₂O (1.2-1.5 equivalents) in water (1 mL/mmol)* and stirred at room temperature until completion of reaction (TLC - complete disappearance of starting material). Methanol was removed under vacuo and the aqueous part was acidified with an aqueous solution of 1N HCl by drop wise addition and simultaneous vigorous stirring. After complete acidification (no more milky white precipitate occurs on addition of dilute HCl), the resulting residue - if it was a solid- was filtered off on a sintered funnel under suction and dried on cellulose filter paper for 10 - 12 h in a dessicator; or -if it was a gum- was extracted with dichloromethane, dried (anhydrwous sodium sulphate) and concentrated under vacuo; to yield the N-cinnamoyl amino acid, usually in good yields as a solid or gum.

*It might be required to use warm water to dissolve LiOH.H₂O in case of larger scale reactions (> 20 mmol). In which case, after dissolving in warm water, it is better to cool the solution before addition to the methanolic solution of the methyl ester.

It is notable that the TLC patterns of most of these N-cinnamoyl peptides with free carboxyl group appear as white streaks, on visualising in iodine chamber.

Synthesis of methyl-N-cinnamoyl-leucinate (16)

To a stirring, ice cold solution of cinnamic acid (1.48 gm, 10mmol) and triethylamine (1.4 mL, 10mmol) in THF (15 mL) was added methylchloroformate (0.77 mL, 10 mmol) and the mixture was stirred vigorously for 2 minutes. After which, a solution of methyl leucinate hydrochloride (2 gm, 10 mmol) in DMSO (4-5 mL) was added followed by triethylamine (3.1 mL, 22 mmol) dissolved in THF (15 mL). The reaction vessel was allowed to warm to room temperature and vigorously stirred for further 3-4 h. Triethylamine hydrochloride was filtered off on a sintered funnel under suction and washed with THF. Removal of solvent from the filtrate under vacuo yielded a residue, which was stirred with saturated aqueous solution of NaHCO_3 (20 mL) for 15 minutes. The bicarbonate layer was decanted and the resulting semi solid washed with water (2X10 mL). An yellow solid precipitated, which was filtered off on a suction funnel and dried in a dessicator under vacuum, on a cellulose filter paper for 10 h. Purification of the resulting solid by column chromatography (EtOAc:Hexane = 1:5.25) (TLC - R_f = 0.5; hexane:ethylacetate 5:1) yielded the above said compound as a crystalline white solid (MP. = 68 °C) in good yields (85%). $[\alpha]_D^{25} = +20^\circ$ ($c = 0.01$, CH_2Cl_2).

^1H NMR, 80 MHz, CDCl_3 , δ 7.73 (d, $J = 8.2\text{Hz}$, 1H), 7.60 (d, $J = 16\text{Hz}$, 1H), 7.35 (s, 3H), 6.70 (d, $J = 16\text{Hz}$, 1H), 4.90 (dd, $J = 10.2\text{Hz}$ & $J = 5.6\text{Hz}$, 1H), 3.82 (s, 3H), 1.73 (dd, $J = 8\text{Hz}$ & $J = 1.6\text{Hz}$, 2H), 1.21 - 1.18 (m, 1H), 0.96 (d, $J = 6.5\text{Hz}$, 6H)

Synthesis of N-cinnamoyl-leucine (17)

To a solution of the methyl-N-cinnamoyl leucinate (1.38 gm, 5 mmol) in MeOH (20 mL) was added a solution of $\text{LiOH}\cdot\text{H}_2\text{O}$ (205 mg, 5 mmol) in water (5 mL) and stirred at room temperature until completion of reaction (TLC - EtOAc:hexane - 1:3 - complete disappearance of starting material). Methanol was removed under vacuo and the aqueous part was acidified with an aqueous solution of 1N HCl by drop wise addition and simultaneous vigorous stirring. After complete acidification (no more milky white precipitate occurs on addition of dilute HCl), the resulting residue was extracted with dichloromethane (30mL), dried (anhydrous sodium sulphate) and concentrated under vacuo to yield N-cinnamoyl-L-leucine, in good yields (85 %) as a hygroscopic gum.

Synthesis of allyl-N-cinnamoyl-leucine amide (15a)

To a stirring, ice-cold solution of N-cinnamoyl-leucine (1.48 gm, 10mmol) and triethylamine (1.4 mL, 10mmol) in THF (15 mL) was added isobutylchloroformate (1.29 mL, 10 mmol) and the mixture was stirred vigorously for 50 - 60 sec. After which, a solution of allyl amine (1.2 mL, 15 mmol) in THF (10 mL) was added followed by triethylamine (2.1 mL, 15 mmol) dissolved in THF (10 mL). The reaction vessel was allowed to warm to room temperature and vigorously stirred for further 3-4 h. Triethylamine hydrochloride was filtered off on a sintered funnel under suction and washed with THF. Removal of solvent from the filtrate under vacuo yielded a residue, which was taken in EtOAc and washed with saturated solution of NaHCO_3 , water and brine. The organic layer was separated, dried (Na_2SO_4) and concentrated in vacuo to yield a residue, which was subjected to column chromatography (Silica gel-EtOAc:Hexane-1:3) (TLC - R_f = 0.5; hexane:ethylacetate 2:1) to yield allyl-N-cinnamoyl-leucine amide as a solid (M.P. = 78-80°C) in good yields (72 %).

^1H NMR, 400 MHz, CDCl_3 , δ , 7.97 (dd, $J = 8.5$ Hz & 1.7 Hz, 1H), 7.79 (t, $J = 5.6$ Hz, 1H), 7.52 (d, $J = 15.9$ Hz, 1H), 7.34 (d, $J = 8$ Hz, 2H), 7.18 - 7.13 (m, 3H), 6.62 (d, $J = 15.9$ Hz, 1H), 5.75 (ddd, $J = 24.3$ Hz, 10.9 Hz & 4.1 Hz, 1H), 5.11 (dd, $J = 15.9$ Hz & 1.2 Hz, 1H), 5.00 (td, $J = 10.3$ Hz & 1.4 Hz, 1H), 4.78 (dd, $J = 13.9$ Hz & 7.9 Hz, 1H), 3.88 (d, $J = 15.9$ Hz, 1H), 3.72 (d, $J = 15.9$ Hz, 1H), 1.68 - 1.57 (m, 2H), 1.17 - 1.44 (m, 1H), 0.84 (ddd, $J = 14.6$ Hz, 5.4 Hz & 3.4 Hz, 6H)

Synthesis of N-cinnamoyl-proline (18)

To an ice cold solution of cinnamoylchloride (1.67 gm, 10 mmol) in dichloromethane (15 mL) was added L-proline (1.15 gm, 10 mmol). To the stirring mixture a solution of triethylamine (3.1 mL, 22 mmol) in dichloromethane (20 mL) was added drop wise at such a rate that the vessel temperature did not exceed 10 °C. After complete addition, the reaction mixture was warmed to room temperature and stirring was continued for 4-5 h. The solvent was evaporated in vacuo. To the resulting residue was added while stirring, aqueous solution of 1N HCl until no more acidification occurred (formation of milky white precipitate ceased). The aqueous suspension of yellowish white solid was filtered on a sintered funnel under suction and dried by spreading on cellulose filter paper for 10 h in a dessicator, to yield N-cinnamoyl proline as a white solid (M.P. = 162 °C) in excellent yields (96 %).

Synthesis of allyl-N-cinnamoyl-proline amide (15b)

To a stirring, ice-cold solution of N-cinnamoyl-proline (2.45 gm, 10mmol) and triethylamine (1.4 mL, 10mmol) in THF (15 mL) was added isobutylchloroformate (1.29 mL, 10 mmol) and the mixture was stirred vigorously for 50 - 60 sec. After which, a solution of allyl amine (1.2 mL, 15 mmol) in THF (10 mL) was added followed by triethylamine (2.1 mL, 15 mmol) dissolved in THF (10 mL). The reaction vessel was allowed to warm to room temperature and vigorously stirred for further 3-4 h. Triethylamine hydrochloride was filtered off on a sintered funnel under suction and washed with THF. Removal of solvent from the filtrate under vacuo yielded a residue, which was taken in EtOAc and washed with saturated solution of NaHCO_3 , water and brine. The organic layer was separated, dried (Na_2SO_4) and concentrated in vacuo to yield a residue, which was subjected to column chromatography (Silica gel-EtOAc:Hexane-1:1.5) (TLC - $R_f = 0.5$; hexane:ethylacetate 1:1) to yield allyl-N-cinnamoyl-proline amide as a solid (M.P. = 92-94 °C) in good yields (81 %).

^1H NMR, 400 MHz, CDCl_3 , δ 7.75 (d, $J = 15.4$ Hz, 1H), 7.56 - 7.53 (m, 2H), 7.42 (bs, 1H), 7.40 - 7.38 (m, 3H), 6.76 (d, $J = 15.4$ Hz, 1H), 5.83 (dq, $J = 10.5$ Hz & 5.4 Hz, 1H), 5.18 (dd, $J = 16.8$ Hz & 5.3 Hz, 1H), 5.11 (d, $J = 10.2$ Hz, 1H), 4.76 (d, $J = 7$ Hz, 1H), 3.87 (dd, $J = 19.3$ Hz & 5.6 Hz, 2H), 3.82 - 3.73 (m, 1H), 3.67 - 3.61 (m, 1H), 2.54 (dd, $J = 12.4$ Hz & 6.6 Hz, 1H), 2.18 (dt, $J = 18.3$ Hz & 9.3 Hz, 1H), 2.08 - 2.04 (m, 1H), 1.91 - 1.81 (m, 1H)

Synthesis of methyl-N-cinnamoyl-proline-leucinate (19c)

A stirring solution of N-cinnamoyl-proline (2.45 gm, 10 mmol) and triethylamine (1.4 mL, 10 mmol) in THF (15 mL) was cooled to -10 °C in an ice-salt bath and to it was added methylchloroformate (0.77 mL, 10 mmol) and stirred vigorously for 50 - 60 seconds. Then a solution of methyl-L-leucinate hydrochloride (2.00 gm, 11 mmol) in DMSO (0.5 mL) was added followed by a solution of triethylamine (3.1 mL, 22 mmol) in THF (15 mL). The mixture was warmed to room temperature by removal of the ice bath and vigorously stirred for further 4 h. Triethylamine hydrochloride was filtered off on a

sintered funnel under suction and washed twice with THF. Removal of solvent from the filtrate under vacuo yielded a residue, which was dissolved in EtOAc (30 mL) and washed with saturated aqueous solution of NaHCO_3 (2X10mL), water (2X10mL) and brine (1X10mL). Drying (Na_2SO_4) and evaporation of solvent under vacuo yielded the crude product which was further purified by column chromatography (EtOAc:Hexane-1:1.5) (TLC - R_f = 0.40; hexane:ethylacetate 1.5:1) to yield methyl-N-cinnamoyl-L-proline-L-leucinate as a solid (M.P. = 110 °C) in good yield (80 %); $[\alpha]_D^{25} = -170.5^\circ$ ($c = 0.01$, CH_2Cl_2).

^1H NMR, 400 MHz, CDCl_3 , δ 7.74 (d, $J = 15.3$ Hz, 1H), 7.66 (d, $J = 7.32$ Hz, 1H), 7.56 - 7.54 (m, 2H), 7.39 - 7.35 (m, 3H), 6.76 (d, $J = 15.6$ Hz, 1H), 4.57 - 4.47 (m, 2H), 3.73 (s, 3H), 3.70 - 3.61 (m, 2H), 2.51 - 2.46 (m, 1H), 2.06 - 2.03 (m, 1H), 2.21 - 2.12 (m, 1H), 1.89 - 1.81 (m, 2H), 1.67 - 1.56 (m, 2H), 0.91 (d, $J = 5.6$ Hz, 3H), 0.88 (d, $J = 5.6$ Hz, 3H); IR (KBr) ν_{max} 3400, 3030, 2950, 2880, 1730, 1640, 1600, 1480, 1440 cm^{-1} ; FT IR (CH_2Cl_2): 3278, 3059, 2956.5, 2872.7, 1744.8, 1649.6, 1598.1, 1542.1, 1498.0, 1425.3

Synthesis of methyl-N-cinnamoyl-proline-phenylalaninate (19d)

A stirring solution of N-cinnamoyl-proline (2.45 gm, 10 mmol) and triethylamine (1.4 mL, 10 mmol) in THF (15 mL) was cooled to -5 °C in an ice-salt bath and to it was added methylchloroformate (0.77 mL, 10 mmol) and stirred vigorously for 50 - 60 seconds. Then a solution of methyl-L-phenylalaninate hydrochloride (2.37 gm, 11 mmol) in DMSO (5 mL) was added followed by a solution of triethylamine (3.1 mL, 11 mmol) in THF (15 mL). The mixture was warmed to room temperature by removal of the ice bath and vigorously stirred for further 4 h. Triethylamine hydrochloride was filtered off on a sintered funnel under suction. Removal of solvent from the filtrate under vacuo yielded a residue, which was stirred with saturated aqueous solution of NaHCO_3 (20mL) for 15 minutes upon which, a solid precipitated. The aqueous bicarbonate layer was decanted and the solid was washed with water (2X10mL) and filtered on a sintered funnel under suction. Drying of the solid on a cellulose filter paper in a desiccator under vacuo yielded the crude product which was further purified by column chromatography (EtOAc:Hexane-1:1.5) (TLC - R_f = 0.40; hexane:ethylacetate 1.5:1) to yield methyl-N-cinnamoyl-L-proline-L-phenylalaninate as a solid (M.P. = 41-43 °C) in good yields (80 %). $[\alpha]_D^{25} = -102^\circ$ ($c=0.004$, CH_2Cl_2).

^1H NMR, 400 MHz, CDCl_3 , δ 7.80 (d, $J = 8$ Hz, 1H), 7.73 (d, $J = 15.4$ Hz, 1H), 7.57 - 7.51 (m, 2H), 7.43 - 7.39 (m, 3H), 7.19 - 7.10 (m, 5H), 6.67 (d, $J = 15.4$ Hz, 1H), 4.84 (dt, $J = 8.1$ Hz & 5.4 Hz, 1H), 4.73 (d, $J = 7.6$ Hz, 1H), 3.73 (s, 3H), 3.54 - 3.51 (m, 2H), 3.19 (dd, $J = 13.9$ Hz & 5.4 Hz, 1H), 2.96 (dd, $J = 13.9$ Hz & 8 Hz, 1H), 2.42 - 2.39 (m, 1H), 1.98 - 1.900 (m, 2H), 1.79 - 1.72 (m, 1H); IR (KBr) ν_{max} 3400- 3300 (br), 3030, 2980, 1760, 1650, 1600 cm^{-1}

Synthesis of N-cinnamoyl-L-proline-L-leucine (20c)

To a solution of the methyl-N-cinnamoyl-proline-leucinate (1.49 gm, 4 mmol) in MeOH (32 mL) was added a solution of $\text{LiOH}\cdot\text{H}_2\text{O}$ (164 mg, 4 mmol) in water (8 mL) and stirred at room temperature until completion of reaction (TLC - EtOAc : hexane - 1 : 1 - complete disappearance of starting material). Methanol was removed under vacuo and the aqueous part was acidified with an aqueous solution of 1N HCl by drop wise addition and simultaneous vigorous stirring. After complete acidification (no more milky white precipitate occurs on addition of dilute HCl), the resulting residue was extracted with

dichloromethane (3X10 mL), dried (anhydrous sodium sulphate) and concentrated under vacuo to yield N-cinnamoyl-L-leucine as a white solid (M.P. = 136-138 °C), in good yields (88 %).

Synthesis of N-cinnamoyl-proline-phenylalanine (20d)

To a solution of the methyl-N-cinnamoyl-proline-phenylalaninate (1.22 gm, 3 mmol) in MeOH (20 mL) was added a solution of LiOH.H₂O (123 mg, 3 mmol) in water (5 mL) and stirred at room temperature until completion of reaction (TLC - EtOAc : hexane - 1 : 1- complete disappearance of starting material). Methanol was removed under vacuo and the aqueous part was acidified with an aqueous solution of 1N HCl by drop wise addition and simultaneous vigorous stirring. After complete acidification (no more milky white precipitate occurred on addition of dilute HCl), the resulting residue was extracted with dichloromethane (3X10mL), dried (anhydrous sodium sulphate) and concentrated under vacuo to yield N-cinnamoyl-phenylalanine as a white solid, in good yields (85 %).

Synthesis of allyl-N-cinnamoyl-proline-leucine amide (15c)

To a stirring, ice-cold solution of N-cinnamoyl-proline-leucine (1.79 gm, 5mmol) and triethylamine (0.7 mL, 5 mmol) in THF (10 mL) was added isobutylchloroformate (0.65 mL, 5 mmol) and the mixture was stirred vigorously for 50 - 60 sec. After which, a solution of allyl amine (0.6 mL, 7.5 mmol) in THF (10 mL) was added followed by triethylamine (1.1 mL, 8 mmol) dissolved in THF (10 mL). The reaction vessel was allowed to warm to room temperature and vigorously stirred for further 3-4 h. Triethylamine hydrochloride was filtered off on a sintered funnel under suction and washed with THF. Removal of solvent from the filtrate under vacuo yielded a residue, which was taken in EtOAc (30 mL) and washed with saturated solution of NaHCO₃ (2X10 mL), water (2X10 mL) and brine (1X10 mL). The organic layer was separated, dried (Na₂SO₄) and concentrated in vacuo to yield a residue, which was subjected to column chromatography (Silica gel-EtOAc:Hexane-3:2) (TLC - R_f = 0.4; hexane:ethylacetate 2:3) to yield allyl-N-cinnamoyl-proline-leucine amide as a solid (M.P. = 135 - 137 °C) in good yields (80 %).

¹H NMR, 400 MHz, CDCl₃, δ, 7.70 (d, J = 15.4 Hz, 1H), 7.52 (d, J = 3.6 Hz, 5H), 7.51(d, J = 1.96 Hz, 0.5 Hz), 7.38 - 7.27 (m, 5H), 7.13 (d, J = 8.1 Hz, 1H), 6.73 (d, J = 15.4 Hz, 1H), 5.81 (tt, J = 11.7 Hz & 5.6 Hz, 1H), 5.15 (dd, J = 1.44 Hz & 17.1 Hz, 1H), 5.09 (dd, J = 7.32 Hz & 1.44 Hz, 1H), 4.66 (dd, J = 8.04 Hz, & 3.4 Hz, 1H), 4.39 - 4.37 (m, 1H), 3.86 - 3.82 (m, 2H), 3.78 (dd, J = 17.6 Hz & 4.4 Hz, 1H), 3.68 (dd, J = 8.1 Hz & 5.2 Hz, 1H), 2.34 - 2.21 (m, 1H), 2.15 - 1.86 (m, 3H), 1.81 - 1.63 (m, 1H), 1.60 - 1.45 (m, 2H), 0.90 (dd, J = 7.5 Hz & 5 Hz, 3H), 0.87 (dd, J = 6.3 Hz & 2.2 Hz, 3H).

Synthesis of allyl-N-cinnamoyl-proline-phenylalanine amide (15d)

To a stirring, ice-cold solution of N-cinnamoyl-proline-phenylalanine (1.96 gm, 15mmol) and triethylamine (0.7 mL, 5 mmol) in THF (10 mL) was added isobutylchloroformate (0.65 mL, 5 mmol) and the mixture was stirred vigorously for 50 - 60 sec. After which, a solution of allyl amine (0.6 mL, 7.5 mmol) in THF (10 mL) was added followed by triethylamine (1.1 mL, 8 mmol) dissolved in THF (10 mL). The reaction vessel was allowed to warm to room temperature and vigorously stirred for further 3-4 h. Triethylamine hydrochloride was filtered off on a sintered funnel under suction and washed with THF. Removal of solvent from the filtrate under vacuo yielded a residue, which was taken in EtOAc (30 mL) and washed with saturated solution of NaHCO₃ (2X10 mL), water (2X10 mL) and brine (1X10 mL). The organic layer was separated,

dried (Na_2SO_4) and concentrated in vacuo to yield a residue, which was subjected to column chromatography (Silica gel-EtOAc:Hexane - 3:2) (TLC - R_f = 0.45; hexane:ethylacetate 2:3) to yield allyl-N-cinnamoyl-proline-phenylalanine amide as a solid (M.P. = 151 - 153 °C) in good yields (81 %).

^1H NMR, 400 MHz, CDCl_3 δ 7.62 (d, J = 15.4 Hz, 1H), 7.55 – 7.53 (m, 2H), 7.43 (d, J = 1.7 Hz, 2H), 7.42 (d, J = 2.1 Hz, 2H), 7.22 – 7.06 (m, 4H), 6.95 (d, J = 8.3 Hz, 1H), 6.67 (bs, 1H), 6.58 (d, J = 15.4 Hz, 1H), 5.81 (ddd, J = 15.9 Hz, 10.5 Hz & 5.6 Hz, 1H), 5.13 (dd, J = 21.8 Hz & 1.2 Hz, 1H), 5.11 (t, J = 1.4 Hz, 1H), 4.70 (dd, J = 3. Hz & 8.3 Hz, 1H), 4.60 (d, J = 5.6 Hz, 1H), 3.88 (tq, J = 15.6 Hz & 5.6 Hz, 1H), 3.60 – 3.49 (m, 2H), 3.20 (dd, J = 14.2 Hz & 5.9 Hz, 1H), 3.11 (dd, J = 13.9 Hz & 8 Hz, 1H), 2.25 – 2.22 (m, 1H), 1.98 – 1.89 (m, 2H), 1.87 – 1.83 (m, 1H)

General Procedure for the PASCOS catalysed Synthesis of Oxirane Containing Peptides from the Corresponding N-cinnamoyl peptides

To a solution of the allyl-N-cinnamoyl-peptide amide (1 equivalent) in CH_3CN (5 mL/mmol) was added 2-methylpropanal (2 equivalents) and PASCOS catalyst[#] (~5 mg) and the contents were stirred under oxygen atmosphere at RT for 12 h. After this time, a fresh lot of the catalyst and 2-methylpropanal (2 equivalents)* were added to the reaction mixture and allowed to stir, until complete conversion of the olefin (TLC).[@] Additional lot of the catalyst and the aldehyde (2 equivalents) were added in periods of 12h until complete conversion of the olefin. The catalyst was filtered off on a sintered funnel and acetonitrile was removed in vacuo. The resulting residue was taken in EtOAc and washed successively with saturated solution of NaHCO_3 , water and brine. Separating the organic phase, drying and concentration in vacuo, yielded the corresponding oxirane containing peptide in crude. These crude epoxides were further subjected to column chromatography (silica gel; EtOAc:Hexane) for purification to get to the corresponding pure epoxides in good yields.

[#] The catalyst should be washed thoroughly with acetonitrile until the wash is constantly colorless. Impurities in the catalyst were found to hinder the formation of epoxide.

* The aldehyde needed to be added in four equivalents for optimum and best conversions. Isobutyraldehyde can also be taken in lots of 3 equivalents and then 1 equivalent. However, lesser reaction times were realised by adding it in lots of 2 equivalents each.

[@] Pre-coated fluorescent silica plates and Silica gel-G coated glass plates were used as immobile phase. TLC plates were visualised in I2 chamber and under U.V. light, (254 nm). Characteristically, all the N-cinnamoyl peptides show up as excellent bright spots under U.V. light, whereas the epoxides show up as very light spots under U.V. light.

Synthesis of allyl-N-(3-phenylglycidyl)-leucine amide (21a)

To a solution of allyl-N-cinnamoyl-leucine amide (450 mg, 1.5 mmol) in CH_3CN (7.5mL) was added 2-methylpropanal (216 mg, 3 mmol) and PASCOS catalyst (~5 mg) and the contents were stirred under oxygen atmosphere at RT for 12 h. After this time, a fresh lot of the catalyst and 2-methylpropanal (216 mg, 3 mmol) were added to the reaction mixture and allowed to stir, until complete conversion of the olefin to the epoxide (TLC - R_f = 0.55; EtOAc:Hexane - 1:2). The catalyst was filtered off on a sintered funnel and acetonitrile was removed in vacuo. The resulting residue was taken in EtOAc (30 mL) and washed with saturated solution of NaHCO_3 (2X10 mL), water (2X10 mL) and brine (1X10 mL). Separating the organic phase, drying and concentration in vacuo, yielded the corresponding oxirane containing peptide in crude. This crude epoxide was further subjected to column chromatography (Silica gel; EtOAc:Hexane - 1:2) for

purification to get to the pure epoxides in excellent yields (85%) as a gum. $[\alpha]_D^{25} = -6^\circ$ ($c = 0.001$, CH_2Cl_2).

^1H NMR, 400 MHz, CDCl_3 , δ 7.57 (dd, $J = 15.9$ Hz, 1H), 7.44 (d, $J = 5.8$ Hz, 1H), 7.32 (bs, 4H), 7.24 - 7.23 (m, 1H), 5.83 - 5.76 (m, 1H), 5.16 (d, $J = 17.1$ Hz, 1H), 5.08 (d, $J = 9.2$ Hz, 1H), 4.67 (bs, 1H), 3.97 & 3.90 (d, $J = 1.96$ Hz, 0.5, 0.5 H), 3.88 (m, 1H), 3.82 (m, 1H), 3.56 & 3.54 (d, $J = 1.96$ Hz, 0.5, 0.5 H), 1.65 - 1.59 (m, 2H), 2.00 - 1.16 (m, 1H), 0.93 (d, $J = 5.12$ Hz, 3H), 0.92 (d, $J = 5.12$ Hz, 3H).

Synthesis of allyl-N-(3-phenylglycidyl)-proline amide (21b)

To a solution of allyl-N-cinnamoyl-proline amide (426 mg, 1.5 mmol) in CH_3CN (7.5 mL) was added 2-methylpropanal (216 mg, 3 mmol) and PASCOS catalyst (~5 mg) and the contents were stirred under oxygen atmosphere at RT for 12 h. After this time, a fresh lot of the catalyst and 2-methylpropanal (216 mg, 3 mmol) were added to the reaction mixture and allowed to stir, until complete conversion of the olefin to the epoxide (TLC - $R_f = 0.45$; EtOAc:Hexane - 1:1). The catalyst was filtered off on a sintered funnel and acetonitrile was removed in vacuo. The resulting residue was taken in EtOAc and washed successively with saturated solution of NaHCO_3 , water and brine. Separating the organic phase, drying and concentration in vacuo, yielded the corresponding oxirane containing peptide in high purity and yields (HPLC). This crude epoxide was further subjected to column chromatography (Silica gel; EtOAc:Hexane - 1:1.5) for purification to get to the pure epoxides in excellent yields (89%) as a gum. $[\alpha]_D^{25} = -46^\circ$ ($c = 0.0015$, CH_2Cl_2).

^1H NMR, 400 MHz, CDCl_3 , δ 7.31 - 7.27 (m, 3H), 7.26 - 7.19 (m, 2H), 6.87 (m, 1H), 5.74 (qd, $J = 27.6$ Hz & 5.4 Hz, 1H), 5.10 (ddd, $J = 15.4$ Hz, 3.4 Hz & 1.8 Hz, 1H), 5.02 (tdd, $J = 10.2$ Hz, 4.9 Hz, 1.4 Hz, 1H), 4.56 (ddd, $J = 13.4$ Hz, 11 Hz & 2.4 Hz, 1H), 4.02, 4.01, 3.99 & 3.98 (d, $J = 1.7$ Hz, 0.4, 0.4, 0.1 & 0.1H), 3.81 - 3.74 (m, 2H), 3.59 (dd, $J = 8.8$ Hz & 5.1 Hz, 1H), 3.54 & 3.52 (d, $J = 1.9$ Hz, 0.5 & 0.5H), 3.45 - 3.41 (m, 1H), 2.39 - 2.27 (m, 1H), 2.10 (dt, $J = 10.5$ Hz & 1.9 Hz, 1H), 1.95 - 1.91 (m, 1H), 1.85 - 1.79 (m, 1H); MS m/z 301 (M^+), 289, 244, 232, 216, 207, 200, 181, 154, 137

Synthesis of allyl-N-(3-phenylglycidyl)-proline-leucine amide (21c)

To a solution of allyl-N-cinnamoyl-proline-leucine amide (595 mg, 1.5 mmol) in CH_3CN (7.5 mL) was added 2-methylpropanal (216 mg, 3 mmol) and PASCOS catalyst (~5 mg) and the contents were stirred under oxygen atmosphere at RT for 12 h. After this time, a fresh lot of the catalyst and 2-methylpropanal (216 mg, 3 mmol) were added to the reaction mixture after 12h and 24h respectively, and allowed to stir, until near complete conversion of the olefin to the epoxide (TLC - $R_f = 0.4$; EtOAc:Hexane - 5:4). The catalyst was filtered off on a sintered funnel and acetonitrile was removed in vacuo. The resulting residue was taken in EtOAc and washed successively with saturated solution of NaHCO_3 , water and brine. Separating the organic phase, drying and concentration in vacuo, yielded the corresponding oxirane containing peptide as a crude residue. This residue was further subjected to flash column chromatography (Silica gel; EtOAc:Hexane - 3:2) for purification to get to the pure epoxides in good yields (62%) as solid (M.P. = 85°C) $[\alpha]_D^{25} = -124.5^\circ$ ($c = 0.018$, CH_2Cl_2).

^1H NMR, 400 MHz, CDCl_3 , δ , 6.47 (d, $J = 4.4$ Hz, 1H), 7.32 - 7.29 (m, 2H), 7.25 - 7.20 (m, 3H), 7.18 - 7.17 (m, 1H), 5.75 (ddd, $J = 15.3$ Hz, 10.2 Hz & 5.6 Hz, 1H), 5.10 (dd, $J = 17.1$ Hz & 1.30 Hz, 1H), 5.04 (d, $J = 10.3$ Hz, 1H), 4.53 (dt, $J = 8.3$ Hz & 5.8 Hz, 1H),

4.36 - 4.31 (m, 1H), 4.01 (d, $J = 1.8$ Hz, 1H), 3.78 (d, $J = 4.4$ Hz, 2H), 3.74 - 3.69 (m, 1H), 3.64 (dd, $J = 7.8$ Hz, & 5.1 Hz, 1H), 3.57 (d, $J = 1.8$ Hz, 0.5 Hz), 3.54 (s, 0.5H), 2.22 - 2.14 (m, 1H), 2.08 - 2.02 (m, 1H), 2.00 - 1.94 (m, 2H), 1.70 - 1.65 (m, 1H), 1.59 - 1.48 (m, 2H), 0.87 (dd, $J = 6.1$ Hz & 3.7 Hz, 3H), 0.83 (d, $J = 5.84$ Hz, 1H); MS m/z 413 (M^+), 391, 341, 307, 289, 268, 228, 209, 181, 154, 136

Synthesis of allyl-N-(3-phenylglycidyl)-proline-phenylalanine amide (21d)

To a solution of allyl-N-cinnamoyl-proline-phenylalanine amide (648 mg, 1.5 mmol) in CH_3CN (7.5 mL) was added 2-methylpropanal (216 mg, 3 mmol) and PASCOS catalyst (~5 mg) and the contents were stirred under oxygen atmosphere at RT for 12 h. After this time, a fresh lot of the catalyst and 2-methylpropanal (216 mg, 3 mmol) were added to the reaction mixture after 12h and 24 h respectively, and allowed to stir, until complete conversion of the olefin to the epoxide (TLC - $R_f = 0.45$; EtOAc:Hexane - 3:2). The catalyst was filtered off on a sintered funnel and acetonitrile was removed in vacuo. The resulting residue was taken in EtOAc and washed successively with saturated solution of $NaHCO_3$, water and brine. Separating the organic phase, drying and concentration in vacuo, yielded the corresponding oxirane containing peptide as a crude residue. This residue was further subjected to flash column chromatography (Silica gel; EtOAc:Hexane - 5:4) for purification to get to the pure epoxide in good yields (64%) as solid (M.P. = 96 °C) $[\alpha]_D^{25} = -96^\circ$ ($c = 0.001$, CH_2Cl_2).

1H NMR, 400 MHz, $CDCl_3$, δ 7.37 - 7.35 (m, 3H), 7.30 - 7.25 (m, 3 H), 7.22 - 7.18 (m, 4H), 7.09 (d, $J = 8.2$ Hz, 1H), 6.91 (t, $J = 5.4$ Hz, 1H), 5.73 (ddd, $J = 22.7$ Hz, 10.3 Hz & 5.4 Hz, 1H), 5.06 (td, $J = 26.2$ Hz & 1.5 Hz, 1H), 5.05 (dd, $J = 2.6$ Hz & 1.4 Hz, 1H), 4.52 (dd, $J = 7.8$ Hz & 3.2 Hz, 1H), 4.00 (d, $J = 2$ Hz, 1H), 3.79 (dd, $J = 7.6$ Hz & 1.7 Hz, 2H), 3.65 - 3.50 (m, 2H), 3.55 (d, $J = 2$ Hz, 1H), 3.23 (dd, $J = 13.9$ Hz & 6.1 Hz, 1H), 3.01 (dd, $J = 13.9$ Hz & 6.1 Hz, 1H), 2.05 - 2.02 (m, 1H), 1.97 - 1.88 (m, 2H), 1.84 - 1.79 (m, 1H); MS m/z 448 (M^+), 141, 328, 302, 244, 216, 200, 154, 136; IR ν_{max} 3300 - 3030 (br), 2880, 1760, 1680, 1660, 1600, 1575

Synthesis of methyl-N-cinnamoyl-proline-leucine-glycinate (22)

A stirring solution of N-cinnamoyl-proline-leucine (1.79 gm, 5 mmol) and triethylamine (0.7 mL, 5 mmol) in THF (10 mL) was cooled to -10 °C in an ice-salt bath and to it was added methylchloroformate (0.38 mL, 5 mmol) and stirred vigorously for 50 - 60 seconds. Then a solution of methyl-L-glycinate hydrochloride (690 mg, 5.5 mmol) in DMSO (1.5 mL) was added followed by a solution of triethylamine (1.6 mL, 11 mmol) in THF (10 mL). The mixture was warmed to room temperature by removal of the ice bath and vigorously stirred for further 4 h. Triethylamine hydrochloride was filtered off on a sintered funnel under suction and washed twice with THF. Removal of solvent from the filtrate under vacuo yielded a residue, which was dissolved in EtOAc (30 mL) and washed with saturated aqueous solution of $NaHCO_3$ (2X10 mL), water (2X10 mL) and brine (1X10 mL). Drying (Na_2SO_4) and evaporation of solvent under vacuo yielded the crude product which was further purified by column chromatography (EtOAc:Hexane-3:2) (TLC - $R_f = 0.45$; hexane:ethylacetate 2:3) to yield methyl-N-cinnamoyl-proline-leucine-glycinate as a gum in good yields (68 %) $[\alpha]_D^{25} = -35.11^\circ$ ($c = 0.014$, CH_2Cl_2).

1H NMR, 400 MHz, $CDCl_3$, δ 7.73 (d, $J = 16.1$ Hz, 1H), 7.53 - 7.50 (m, 2H), 7.37 - 7.25 (m, 3H), 7.34 (d, $J = 7.8$ Hz, 1H), 7.19 (t, $J = 5.6$ Hz, 1H), 4.71 (d, $J = 8$ Hz, 1H), 4.47 - 4.45 (m, 1H), 4.10 dd, $J = 12.2$ Hz & 4.5 Hz, 1H), 3.95 (dd, $J = 12.2$ Hz & 4.5 Hz, 1H),

3.81 - 3.78 (m, 1H), 3.71 (s, 3H), 3.69 - 3.65 (m, 1H), 2.45 - 2.41 (m, 1H), 2.23 - 1.96 (m, 3H).

Synthesis of N-cinnamoyl-proline-leucine-glycine (23)

To a solution of the methyl-N-cinnamoyl-proline-leucine-glycinate (1.29 gm, 3 mmol) in MeOH (16 mL) was added a solution of LiOH.H₂O (143 mg, 3.5 mmol) in water (4 mL) and stirred at room temperature until completion of reaction (TLC - EtOAc:hexane - 3:1 - complete disappearance of starting material). Methanol was removed under vacuo and the aqueous part was acidified with an aqueous solution of 1N HCl by drop wise addition and simultaneous vigorous stirring. After complete acidification (no more milky white precipitate occurred on addition of dilute HCl), the resulting residue was extracted with dichloromethane (3X10 mL), dried (anhydrous sodium sulphate) and concentrated under vacuo to yield N-cinnamoyl-proline-leucine-glycine, in good yields (75 %) as a gum.

Synthesis of allyl-N-cinnamoyl-proline-leucine-glycinate (15e)

To a solution of N-cinnamoyl-proline-leucine-glycine (830 mg, 2 mmol) in acetone (15 mL) was added K₂CO₃ (455 mg, 3.3 mmol) and allylbromide (268 mg, 2.2 mmol) and the reaction mixture was set to reflux for 8 h during which time, the reaction was almost complete. The inorganic salts were filtered off on a sintered funnel under suction and solvent was removed under vacuo. The resulting residue was taken in EtOAc (30 mL) and washed with saturated aqueous solution of NaHCO₃ (2X10 mL), water (2X10 mL) and brine (1X10 mL). Drying the organic layer (anhydrous Na₂SO₄) and concentration in vacuo yielded a thick residue which was purified by column chromatography (EtOAc in Hexane-50 %) (TLC - R_f = 0.5; hexane : ethylacetate 2:3) to yield allyl-N-cinnamoyl-proline-leucine-glycinate as a crystalline solid (M.P = 52-54 °C), in good yields (72 %) [α]_D²⁵ = -93.43 ° (c = 0.0105, CH₂Cl₂).

¹H NMR, 400 MHz, CDCl₃, δ 7.72 (d, J = 15.4 Hz, 1H), 7.56 - 7.53 (m, 2H), 7.41 - 7.34 (m, 3H), 7.31 (d, J = 8 Hz, 1H), 7.21 - 7.19 (m, 1H), 6.77 (d, J = 15.5 Hz, 1H), 7.21 - 7.19 (m, 1H), 6.77 (d, J = 15.5 Hz, 1H), 5.88 (ddd, J = 17.1 Hz, 10.5 Hz & 5.8 Hz, 1H), 5.31 (td, J = 15.7 Hz & 1.5 Hz, 1H), 5.23 (td, J = 9.3 Hz & 1.2 Hz, 1H), 4.71 (dd, J = 8.1 Hz & 3 Hz, 1H), 4.60 (td, J = 5.9 Hz & 1.2 Hz, 2H), 4.48 - 4.45 (m, 1H), 4.14 (dd, J = 18.1 Hz & 5.9 Hz, 1H), 3.97 (dd, J = 18.1 Hz & 5.5 Hz, 1H), 3.81 - 3.79 (m, 1H), 3.70 (dd, J = 8.8 Hz & 7 Hz, 1H), 2.36 (td, J = 10.8 Hz & 3.2 Hz, 1H), 2.14 - 1.99 (m, 3H), 1.58 (ddd, J = 25.6 Hz, 10 Hz & 5.4 Hz, 2H), 0.89 (t, J = 6.1 Hz, 3H), 0.87 (dd, J = 6.4 Hz & 3.6 Hz, 3H).

Synthesis of allyl-N-(3-phenylglycidyl)-proline-leucine-glycinate (21e)

To a solution of allyl-N-cinnamoyl-proline-leucine glycinate (546 mg, 1.2 mmol) in CH₃CN (6 mL) was added 2-methylpropanal (173 mg, 2.4 mmol) and PASCOS catalyst (~5 mg) and the contents were stirred under oxygen atmosphere at RT for 12 h. After this time, a fresh lot of the catalyst and 2-methylpropanal (173 mg, 2.4 mmol) were added to the reaction mixture and allowed to stir, until complete conversion of the olefin to the epoxide (TLC - R_f = 0.4; EtOAc:Hexane - 3:2). The catalyst was filtered off on a sintered funnel and acetonitrile was removed in vacuo. The resulting residue was taken in EtOAc and washed successively with saturated solution of NaHCO₃, water and brine. Separating the organic phase, drying and concentration in vacuo, yielded the corresponding oxirane containing peptide as a crude residue. This crude residue was further subjected to column chromatography (Silica gel; EtOAc:Hexane 60%) for purification to get to the pure epoxides in poor yields (~10%) as a gum [α]_D²⁵ = -28.8 ° (c = 0.005, CH₂Cl₂).

^1H NMR, 400 MHz, CDCl_3 , δ 7.65 (d, J = 8.8 Hz, 1H), 7.66 - 7.23 (m, 5H), 7.02 (bs, 1H), 5.88 (qd, J = 15.8 Hz & 5.6 Hz, 1H), 5.29 (dd, J = 12 Hz & 1.5 Hz, 1H), 5.22 (dd, J = 10 Hz & 1.2 Hz, 1H), 4.71 (dd, J = 8 Hz & 4.4 Hz, 1H), 4.59 (td, J = 6 Hz & 1.2 Hz, 1H), 4.47 - 4.44 (m, 1H), 4.22 (dd, J = 11.2 Hz & 5.1 Hz, 1H), 4.16 (dd, J = 11.2 Hz & 5.1 Hz, 1H), 4.10 (s, 1H), 3.98 - 3.91 (m, 1H), 3.78 - 3.69 (m, 1H), 3.61 (s, 1H), 2.28 - 2.46 (m, 1H), 2.14 - 1.94 (m, 3H), 1.82 - 1.73 (m, 1H), 1.65 - 1.56 (m, 2H), 0.95 - 0.85 (m, 6H); IR ν_{max} 3030, 2980, 2870, 1775, 1760, 1680, 1600, 1560 cm^{-1}

Synthesis of methyl-4-(N-cinnamoyl-L-leucinyl)-crotonate (15h)

To a solution of N-cinnamoyl-L-leucine (1.35 gm, 5 mmol) in acetone (25 mL) was added K_2CO_3 (828 mg, mmol) and allylbromide (1.08 gm, 6 mmol) and the reaction mixture was set to reflux for 8 h during which time, the reaction was almost complete. The inorganic salts were filtered off on a sintered funnel under suction and solvent was removed under vacuo. The resulting residue was taken in EtOAc (30 mL) and washed with saturated aqueous solution of NaHCO_3 (2X10 mL), water (2X10 mL) and brine (1X10 mL). Drying the organic layer (anhydrous Na_2SO_4) and concentration in vacuo yielded a thick residue which was purified by column chromatography (EtOAc in Hexane-25 %) (TLC - R_f = 0.5; hexane : ethylacetate 2.5:1) to yield methyl-4-(N-cinnamoyl-L-leucinyl)-crotonate as a white solid (M.P. 78-80 $^\circ\text{C}$), in good yields (62%).

^1H NMR, 400 MHz, CDCl_3 , δ 7.69 (d, J = 16 Hz, 1H), 7.44 - 7.22 (m, 5H), 6.98 (td, J = 20 Hz & 5.6 Hz, 1H), 6.56 (d, J = 16 Hz, 1H), 6.09 (td, J = 20 Hz & 3 Hz, 1H), 4.83 (dd, J = 6.4 Hz & 3 Hz, 2H), 3.76 (s, 3H), 1.65 (dd, J = 6.5 Hz & 3 Hz, 2H), 1.20 - 1.13 (m, 1H), 0.92 (d, J = 6.6 Hz, 6H)

Synthesis of methyl-4-(N-cinnamoyl-L-proline-L-leucinyl)-crotonate (15i)

To a solution of N-cinnamoyl-L-proline-L-leucine (537 mg, 1.5 mmol) in acetone (7.5 mL) was added K_2CO_3 (228 mg, 1.65 mmol) and allylbromide (mL, mmol) and the reaction mixture was set to reflux for 8 h during which time, the reaction is almost complete. The inorganic salts were filtered off on a sintered funnel under suction and solvent was removed under vacuo. The resulting residue was taken in EtOAc (20 mL) and washed with saturated aqueous solution of NaHCO_3 (2X10 mL), water (2X10 mL) and brine (1X10 mL). Drying the organic layer (anhyd. Na_2SO_4) and concentration in vacuo yielded a thick residue which was purified by column chromatography (EtOAc in Hexane-40 %) (TLC - R_f = 0.5; hexane : ethylacetate 1:1) to yield methyl-4-(N-cinnamoyl-L-proline-L-leucinyl)-crotonate as a white solid, (M.P = 92 $^\circ\text{C}$) in good yields (68 %).

^1H NMR, 400 MHz, CDCl_3 , δ 7.79 (d, J = 7.3 Hz, 1H), 7.75 (d, J = 15.5 Hz, 1H), 7.56 - 7.53 (m, 2H), 7.41 - 7.35 (m, 3H), 6.94 (td, J = 15.9 Hz & 1.7 Hz, 1H), 6.76 (d, J = 15.4 Hz, 1H), 6.08 (td, J = 15.9 Hz & 1.7 Hz, 1H), 4.79 (dd, J = 4.9 Hz & 1.9 Hz, 2H), 4.64 (d, J = 3.2 Hz, 1H), 4.51 (dd, J = 12.9 Hz & 5.6 Hz, 1H), 3.79 - 3.76 (m, 1H), 3.75 (s, 3H), 3.62 (dd, J = 16.8 Hz & 9.5 Hz, 1H), 2.53 - 2.48 (m, 1H), 2.29 - 2.28 (m, 1H), 2.11 - 2.07 (m, 1H), 1.91 - 1.81 (m, 1H), 1.70 - 1.62 (m, 2H), 1.27 - 1.24 (m, 1H), 0.93 (d, J = 5.8 Hz, 3H), 0.89 (d, J = 5.8 Hz, 3H)

Synthesis of methyl-4-(N-(3-phenylglycidyl)leucinyl)-crotonate (21h)

To a solution of methyl-4(N-cinnamoyl-leucinyl) crotonate (684 mg, 1.5 mmol) in CH_3CN (7.5 mL) was added 2-methylpropanal (216 mg, 3 mmol) and PASCOS catalyst

(~5 mg) and the contents were stirred under oxygen atmosphere at RT for 12 h. After this time, a fresh lot of the catalyst and 2-methylpropanal (216 mg, 3 mmol) were added to the reaction mixture and allowed to stir, until complete conversion of the olefin to the epoxide (TLC - R_f = 0.45; EtOAc:Hexane - 1:1). The catalyst was filtered off on a sintered funnel and acetonitrile was removed in vacuo. The resulting residue was taken in EtOAc (20 mL) and washed successively with saturated solution of NaHCO_3 (2X10 mL), water (2X10 mL) and brine (1X10 mL). Separating the organic phase, drying and concentration in vacuo, yielded the corresponding oxirane containing peptide as a crude residue. This residue was further subjected to flash column chromatography (Silica gel; EtOAc:Hexane - 5:6) for purification to get to the pure epoxide in excellent yields (90%) as a gum $[\alpha]_D^{25} = -8^\circ$ ($c = 0.001$, CH_2Cl_2).

^1H NMR, 400 MHz, CDCl_3 , δ 7.33 - 7.32 (m, 4H), 7.25 - 7.23 (m, 2H), 6.97 - 6.90 (m, 2H), 6.05 (d, $J = 15.8$ Hz, 1H), 4.77 (dd, $J = 4.7$ Hz & 2.8 Hz, 2H), 4.68 (dd, $J = 9.5$ Hz & 8.1 Hz, 1H), 3.98 & 3.90 (d, $J = 1.4$ Hz, 1H), 3.73 & 3.72 (s, 3H), 3.58 & 3.55 (d, $J = 1.4$ Hz, 1H), 1.68 (dt, 14.4 Hz & 5.6 Hz, 2H), 1.17 - 1.09 (m, 1H), 0.96 (d, $J = 5.1$ Hz); MS m/z 376, 360, 318, 303, 278, 260, 228, 184, 154

Synthesis of methyl-4-(N-(3-phenylglycidyl)proline-leuciny)-crotonate (21i)

To a solution of methyl-4-(N-cinnamoyl-leuciny) crotonate (538 mg, 1.5 mmol) in CH_3CN (5 mL/mmol) was added 2-methylpropanal (216 mg, 3mmol) and PASCOS catalyst (~5 mg) and the contents were stirred under oxygen atmosphere at RT for 12 h. After this time, a fresh lot of the catalyst and 2-methylpropanal (216 mg, 3mmol) were added to the reaction mixture and allowed to stir, until complete conversion of the olefin to the epoxide (TLC - R_f = 0.45; EtOAc:Hexane - 1:2.5). The catalyst was filtered off on a sintered funnel and acetonitrile was removed in vacuo. The resulting residue was taken in EtOAc(20 mL) and washed successively with saturated solution of NaHCO_3 (2X10 mL), water (2X10 mL) and brine (1X10 mL). Separating the organic phase, drying and concentration in vacuo, yielded the corresponding oxirane containing peptide as a crude residue. This crude residue was further subjected to column chromatography (Silica gel; EtOAc:Hexane - 1:2) for purification to get to the pure epoxide in excellent yields (90%) as gum $[\alpha]_D^{25} = -108^\circ$ ($c = 0.010$, CH_2Cl_2).

^1H NMR, 400 MHz, CDCl_3 , δ , 7.32 - 7.27 (m, 2H), 7.25 - 7.20 (m, 3H), 6.87 (td, $J = 15.6$ Hz & 4.6 Hz, 1H), 5.98 (dd, $J = 15.9$ Hz & 2 Hz, 1H), 4.72 (dd, $J = 4.6$ Hz & 1.7 Hz, 2H), 4.61 (d, $J = 8$ Hz, 1H), 4.46 (t, $J = 7.2$ Hz, 1H), 3.99 (d, $J = 1.7$ Hz, 1H), 3.69 (s, 3H), 3.37 (m, 1H), 3.61 (dd, $J = 9$ Hz & 5.4 Hz, 1H), 3.54 (d, $J = 1.7$ Hz, 1H), 2.35 - 2.29 (m, 1H), 2.12 - 2.07 (m, 1H), 1.96 - 1.95 (m, 1H), 1.89 - 1.84 (m, 1H), 1.60 - 1.54 (m, 2H), 1.86 (t, $J = 7.3$ Hz, 1H), 0.91 (d, $J = 4.6$ Hz, 3H), 0.87 (d, $J = 4.6$ Hz, 3H); MS m/z 473 (M^+), 391, 353, 325, 307, 289, 244, 216, 200, 181, 154

General Procedure for the PASCOS Catalysed Epoxidation of N-cinnamoyl-peptides in the Presence of NaOAc: Method-B

To a solution of the N-cinnamoyl-peptide (1 equivalent) in CH_3CN (5 mL/mmol) was added 2-methylpropanal (2 equivalents), NaOAc (8 equivalents) and PASCOS catalyst (~5 mg) and the contents were stirred under oxygen atmosphere at RT for 12 h by which time the conversion is almost complete. After this time, a fresh lot of the catalyst and 2-methylpropanal (1 equivalent) were added to the reaction mixture and allowed to stir, if needed, until complete conversion of the olefin (TLC). The catalyst and inorganic salts

were filtered off on a sintered funnel and acetonitrile was removed in vacuo. The resulting residue was taken in EtOAc and washed successively with saturated solution of NaHCO_3 , water and brine. Separating the organic phase, drying and concentration in vacuo, yielded the corresponding oxirane containing peptide in high purity and yields (HPLC). These epoxides were further subjected to column chromatography (Silica gel; EtOAc:Hexane) for purification to get to the corresponding epoxides in excellent yields.

Synthesis of allyl-N-(3-phenylglycidyl)-proline amide (Method-B) (21b)

To a solution of the allyl-N-cinnamoyl-proline amide (142 mg, 0.5 mmol) in CH_3CN (5 mL) was added 2-methylpropanal (72 mg, 1 mmol), NaOAc (328 mg, 4 mmol) and PASCOS catalyst (~5 mg) and the contents were stirred under oxygen atmosphere at RT for 15 h. After this the catalyst and inorganic salts were filtered off on a sintered funnel and acetonitrile was removed in vacuo. The resulting residue was taken in EtOAc and washed successively with saturated solution of NaHCO_3 , water and brine. Separating the organic phase, drying and concentration in vacuo, yielded the corresponding oxirane containing peptide as a crude residue (HPLC). These epoxides were further subjected to column chromatography (Silica gel; EtOAc:Hexane - 1:1.86) for purification to get to the corresponding epoxide in excellent yields (92%) $[\alpha]_{\text{D}}^{25} = -42^\circ$ ($c = 0.01$, CH_2Cl_2).

^1H NMR, 400 MHz, CDCl_3 , δ 7.31 - 7.27 (m, 3H), 7.26 - 7.19 (m, 2H), 6.87 (m, 1H), 5.74 (qd, $J = 27.6$ Hz & 5.4 Hz, 1H), 5.10 (ddd, $J = 15.4$ Hz, 3.4 Hz & 1.8 Hz, 1H), 5.02 (tdd, $J = 10.2$ Hz, 4.9 Hz, 1.4 Hz, 1H), 4.56 (ddd, $J = 13.4$ Hz, 11 Hz & 2.4 Hz, 1H), 4.02, 4.01, 3.99 & 3.98 (d, $J = 1.7$ Hz, 0.4, 0.4, 0.1 & 0.1H), 3.81 - 3.74 (m, 2H), 3.59 (dd, $J = 8.8$ Hz & 5.1 Hz, 1H), 3.54 & 3.52 (d, $J = 1.9$ Hz, 0.5 & 0.5H), 3.45 - 3.41 (m, 1H), 2.39 - 2.27 (m, 1H), 2.10 (dt, $J = 10.5$ Hz & 1.9 Hz, 1H), 1.95 - 1.91 (m, 1H), 1.85 - 1.79 (m, 1H); MS m/z 301 (M^+), 289, 244, 232, 216, 207, 200, 181, 154, 137

Synthesis of allyl-N-(3-phenylglycidyl)-proline-leucine amide (Method-B) (21c)

To a solution of the allyl-N-cinnamoyl-proline-leucine amide (595 mg, 1.5 mmol) in CH_3CN (7.5 mL) was added 2-methylpropanal (216 mg, 3 mmol), NaOAc (656 mg, 8 mmol) and PASCOS catalyst (~5 mg) and the contents were stirred under oxygen atmosphere at RT for 12 h. After this time, a fresh lot of the catalyst and 2-methylpropanal (103 mg, 1.5 mmol) were added to the reaction mixture and allowed to stir, until complete conversion (20 h) of the olefin (TLC). The catalyst and inorganic salts were filtered off on a sintered funnel and acetonitrile was removed in vacuo. The resulting residue was taken in EtOAc and washed successively with saturated solution of NaHCO_3 , water and brine. Separating the organic phase, drying and concentration in vacuo, yielded the corresponding oxirane containing peptide as a crude residue. This crude residue was further subjected to column chromatography (Silica gel; EtOAc:Hexane - 3:2) for purification to get to the corresponding epoxides in excellent yields (89%) $[\alpha]_{\text{D}}^{25} = -116^\circ$ ($c = 0.002$, CH_2Cl_2).

^1H NMR, 400 MHz, CDCl_3 , δ , 6.47 (d, $J = 4.4$ Hz, 1H), 7.32 - 7.29 (m, 2H), 7.25 - 7.20 (m, 3H), 7.18 - 7.17 (m, 1H), 5.75 (ddd, $J = 15.3$ Hz, 10.2 Hz & 5.6 Hz, 1H), 5.10 (dd, $J = 17.1$ Hz & 1.30 Hz, 1H), 5.04 (d, $J = 10.3$ Hz, 1H), 4.53 (dt, $J = 8.3$ Hz & 5.8 Hz, 1H), 4.36 - 4.31 (m, 1H), 4.01 (d, $J = 1.8$ Hz, 1H), 3.78 (d, $J = 4.4$ Hz, 2H), 3.74 - 3.69 (m, 1H), 3.64 (dd, $J = 7.8$ Hz, & 5.1 Hz, 1H), 3.57 (d, $J = 1.8$ Hz, 0.5 Hz), 3.54 (s, 0.5H), 2.22 - 2.14 (m, 1H), 2.08 - 2.02 (m, 1H), 2.00 - 1.94 (m, 2H), 1.70 - 1.65 (m, 1H), 1.59 - 1.48

(m, 2H), 0.87 (dd, $J = 6.1$ Hz & 3.7 Hz, 3H), 0.83 (d, $J = 5.84$ Hz, 1H); MS m/z 413 (M^+), 391, 341, 307, 289, 268, 228, 209, 181, 154, 136

Synthesis of allyl-N-(phenylglycidyl)-proline-phenylalanine amide (Method-B) (21d)

To a solution of the allyl-N-cinnamoyl-proline-phenylalanine amide (648 mg, 1.5 mmol) in CH_3CN (7.5 mL) was added 2-methylpropanal (216 mg, 3 mmol), NaOAc (984 mg, 12 mmol) and PASCOS catalyst (~5 mg) and the contents were stirred under oxygen atmosphere at RT for 12 h. After this time, a fresh lot of the catalyst and 2-methylpropanal (103 mg, 1.5 mmol) were added to the reaction mixture and allowed to stir, until complete conversion (17 h) of the olefin (TLC). The catalyst and inorganic salts were filtered off on a sintered funnel and acetonitrile was removed in vacuo. The resulting residue was taken in EtOAc and washed successively with saturated solution of NaHCO_3 , water and brine. Separating the organic phase, drying and concentration in vacuo, yielded the corresponding oxirane containing peptide as a crude residue. This crude residue was further subjected to column chromatography (Silica gel; EtOAc:Hexane - 5:4) for purification to get to the corresponding epoxide in excellent yields (93%) $[\alpha]_D^{25} = -100^\circ$ ($c = 0.009$, CH_2Cl_2).

^1H NMR, 400 MHz, CDCl_3 , δ 7.37 – 7.35 (m, 3H), 7.30 – 7.25 (m, 3 H), 7.22 – 7.18 (m, 4H), 7.09 (d, $J = 8.2$ Hz, 1H), 6.91 (t, $J = 5.4$ Hz, 1H), 5.73 (ddd, $J = 22.7$ Hz, 10.3 Hz & 5.4 Hz, 1H), 5.06 (td, $J = 26.2$ Hz & 1.5 Hz, 1H), 5.05 (dd, $J = 2.6$ Hz & 1.4 Hz, 1H), 4.52 (dd, $J = 7.8$ Hz & 3.2 Hz, 1H), 4.00 (d, $J = 2$ Hz, 1H), 3.79 (dd, $J = 7.6$ Hz & 1.7 Hz, 2H), 3.65 – 3.50 (m, 2H), 3.55 (d, $J = 2$ Hz, 1H), 3.23 (dd, $J = 13.9$ Hz & 6.1 Hz, 1H), 3.01 (dd, $J = 13.9$ Hz & 6.1 Hz, 1H), 2.05 – 2.02 (m, 1H), 1.97 – 1.88 (m, 2H), 1.84 – 1.79 (m, 1H); IR ν_{max} 3300 – 3030 (br), 2880, 1760, 1680, 1660, 1600, 1575

Synthesis of methyl-N-(3-phenylglycidyl)-proline-leucinate (Method-B) (21f)

To a solution of the methyl-N-cinnamoyl-proline leucinate (558 mg, 1.5 mmol) in CH_3CN (7.5 mL) was added 2-methylpropanal (216 mg, 3 mmol), NaOAc (984 mg, 12 mmol) and PASCOS catalyst (~5 mg) and the contents were stirred under oxygen atmosphere at RT for 14 h until complete conversion of the olefin (TLC). The catalyst and inorganic salts were filtered off on a sintered funnel and acetonitrile was removed in vacuo. The resulting residue was taken in EtOAc and washed successively with saturated solution of NaHCO_3 , water and brine. Separating the organic phase, drying and concentration in vacuo, yielded the corresponding oxirane containing peptide as a crude residue. This crude residue was further subjected to flash column chromatography (Silica gel; EtOAc:Hexane - 1:1.5) for purification to get to the corresponding epoxide in excellent yields(90%) as a solid. $[\alpha]_D^{25} = -118^\circ$ ($c = 0.013$, CH_2Cl_2).

^1H NMR 400 MHz, CDCl_3 δ 7.34 - 7.20 (m, 5 H), 7.15 (d, $J = 7.9$ Hz, 1H), 4.67 (d, $J = 5.6$ Hz, 1H), 4.49 (t, $J = 4.2$ Hz, 1H), 4.03 (d, $J = 3.6$ Hz, 1H), 3.73 (s, 3H), 3.61 (d, $J = 3.6$ Hz, 1H), 3.57 (m, 2H), 2.39 - 1.89 (m, 1H), 0.93 (dd, $J = 6.6$ Hz, 6H); MS m/z 389, 339, 307, 281, 269, 244, 216, 209, 181, 154, 136; IR ν_{max} 3200 (br), 3030, 2910, 2880, 1760, 1655 cm^{-1}

Synthesis of methyl-N-(3-phenylglycidyl)-proline-aspartate (Method-B) (21g)

To a solution of the methyl-N-cinnamoyl-proline-aspartate (78 mg, 0.2 mmol) in CH_3CN (2 mL) was added 2-methylpropanal (30 mg, 0.4 mmol), NaOAc (131 mg, 1.6 mmol) and

PASCOS catalyst (~5 mg) and the contents were stirred under oxygen atmosphere at RT for 14 h until complete conversion of the olefin (TLC). The catalyst and inorganic salts were filtered off on a sintered funnel and acetonitrile was removed in vacuo. The resulting residue was taken in EtOAc and washed successively with saturated solution of NaHCO_3 , water and brine. Separating the organic phase, drying and concentration in vacuo, yielded the corresponding oxirane containing peptide as a crude residue which was further subjected to flash column chromatography (Silica gel; EtOAc:Hexane - 1:1) for purification to get to the corresponding epoxide as a gum in excellent yields(89%).

^1H NMR, 400 MHz, CDCl_3 , δ 7.52(d, J = 8.3 Hz, 1H), 7.35 – 7.32 (m, 5H), 4.85 (dd, J = 8 Hz & 4.1 Hz, 1H), 4.62 (t, J = 3.6 Hz, 1H), 4.14 & 4.08 (d, J = 5.7 Hz, 0.5H each), 3.82 (dt, J = 9.8 Hz & 4.2 Hz, 1H), 3.74 (s, 3H), 3.69 (s, 3H), 3.65 – 3.61 (m, 1H), 3.58 (d, J = 5.7 Hz, 1H), 2.92 (dd, J = 20 Hz & 15.1 Hz, 2H), 2.36 – 2.33 (m, 1H), 2.27 – 2.19 (m, 1H), 2.15 – 2.10 (m, 1H), 1.90 (bs, 1H)

General Procedure for the Synthesis of Azido Alcohol Containing Peptides from Oxirane Containing Peptides

To a solution of the oxirane containing peptide (1 equivalent) in methanol (8 mL/mmol) was added NaN_3 (5 equivalents) and NH_4Cl (2.2 equivalents) followed by water (1 mL/mmol) and the reaction mixture was refluxed for 8 h. The solvent was removed to give a residue which was taken in EtOAc (10 mL/mmol) and washed with water (2X5 mL) and the organic layer was dried (Na_2SO_4) and concentrated in vacuum to yield a residue which was purified by subjection to column chromatography (Silica gel-EtOAc:hexane) to yield the corresponding azido alcohol in good yields as a mixture of regio-isomers. No efforts were made to separate the regio-isomers. The azido alcohols show as dark spots on TLC when visualised in I_2 chamber. But look very light when visualised under U.V light 25 nm.

Synthesis of azido alcohol containing peptide (25a)

To a solution of the oxirane containing peptide (**21a**) (316 mg, 1 mmol) in methanol (8 mL) was added NaN_3 (325 mg, 5 mmol) and NH_4Cl (118 mg, 2.2 mmol) followed by water (1 mL/mmol) and the reaction mixture was refluxed for 8 h. The solvent was removed to give a residue which was taken in EtOAc (10 mL/mmol) and washed with water (2X5 mL) and the organic layer was dried (Na_2SO_4) and concentrated in vacuum to yield a residue which was purified by subjection to column chromatography (Silica gel-EtOAc:hexane - 1:2) to yield the corresponding azido alcohol in good yields (86%) as a mixture of regio-isomers as a gum.

^1H NMR, 400 MHz, CDCl_3 , δ 7.39 - 7.34 (m, 5H), 6.82 (d, J = 8.6 Hz, 1H), 6.38 (t, J = 5.5 Hz, 1H), 5.79 (ddd, J = 22.7 Hz, 11Hz & 5.6 Hz, 1H), 5.16 (dd, J = 23.6 Hz & 1.5 Hz, 1H), 5.15 (d, J = 1.2 Hz, 1H), 5.01 (d, J = 3.6 Hz, 1H), 4.50 (t, J = 4 Hz, 1H), 4.24 (dd, J = 9.3 Hz & 4 Hz, 1H), 3.85 - 3.81 (m, 2H), 1.81 (bs, 1H), 1.38 (td, J = 19 Hz & 4.1 Hz, 1H), 1.27 (td, J = 19 Hz & 4.1 Hz, 1H), 0.94 - 0.86 (m, 1H), 0.73 (d, J = 6.6 Hz, 3H), 0.66 (d, J = 6.6 Hz, 3H); IR ν_{max} 3340, 3060, 3040, 2110, 1750, 1600 cm^{-1} .

Synthesis of azido alcohol containing peptide (25b)

To a solution of the oxirane containing peptide **21b** (426 mg, 1.5 mmol) in methanol (12 mL) was added NaN_3 (487 mg, 7.5 mmol) and NH_4Cl (177 mg, 3.3 mmol) followed by water (1.5 mL) and the reaction mixture was refluxed for 8 h. The solvent was removed

Synthesis of azido alcohol containing peptide (25e)

To a solution of the oxirane containing peptide **21e** (398 mg, 1 mmol) in methanol (8 mL) was added NaN_3 (325 mg, 5 mmol) and NH_4Cl (118 mg, 2.2 mmol) followed by water (1 mL) and the reaction mixture was refluxed for 8 h. The solvent was removed to give a residue which was taken in EtOAc (10 mL/mmol) and washed with water (2X5 mL) and the organic layer was dried (Na_2SO_4) and concentrated in vacuum to yield a residue which was purified by subjection to flash column chromatography (Silica gel-EtOAc:hexane - 3:2) to yield the corresponding azido alcohol in good yields (83 %) as a mixture of regio-isomers as a gum $[\alpha]_{\text{D}}^{25} = -71.1^\circ$ ($c = 0.003$, CH_2Cl_2).

^1H NMR, 400 MHz, CDCl_3 , δ 7.42 - 7.29 (m, 5H), 7.22 (d, $J = 8.6$ Hz, 1H), 4.80 & 4.74 (d, $J = 7.8$ Hz, 1H), 4.66 - 4.61 (m, 1H), 4.59 - 4.57 (m, 1H), 4.40 & 4.38 (d, $J = 7.8$ Hz, 1H), 3.70 (s, 3H), 3.65 - 3.49 (m, 2H), 2.33 - 2.24 (m, 1H), 2.03 - 1.89 (m, 2H), 1.67 - 1.48 (m, 3H), 1.26 - 1.22 (m, 1H), 0.94 (t, $J = 5.6$ Hz, 3H), 0.89 (dd, $J = 13.6$ Hz & 6.3 Hz, 3H); IR ν_{max} 3340, 3040, 2880, 2100 (s), 1740, 1675, 1600, 1550 cm^{-1}

General Procedure for the synthesis of Aziridine Containing Peptides From Azido Alcohol containing Peptides

To a solution of the azido alcohol (1 equivalent) in CH_3CN (8 mL/mmol) was added triphenylphosphine (1 equivalent) in portions, with vigorous stirring at ambient conditions. The reaction mixture was stirred for a further 0.5 h at ambient temperature after complete addition of PPh_3 ; after which, the solution was set to reflux for 6 h. The solvent was evaporated under vacuum and the resulting residue was subjected to flash column chromatography (Silica gel - EtOAc:Hexane) to yield the corresponding aziridine containing peptide in good yields.

Synthesis of aziridine containing peptide (26a)

To a solution of the azido alcohol **25a** (215 mg, 0.6 mmol) in CH_3CN (4.8 mL) was added triphenylphosphine (158 mg, 0.6 mmol) in portions, with vigorous stirring at ambient conditions. The reaction mixture was stirred for a further 0.5 h at ambient temperature after complete addition of PPh_3 ; after which, the solution was set to reflux for 6 h. The solvent was evaporated under vacuum and the resulting residue was subjected to flash column chromatography (Silica gel - EtOAc:Hexane - 1:2.1) to yield the corresponding aziridine containing peptide in poor yields (8%) as a gum, $[\alpha]_{\text{D}}^{25} = -0.5^\circ$ ($c = 0.001$, CH_2Cl_2).

^1H NMR, 400 MHz, CDCl_3 , δ 7.29 - 7.19 (m, 5H), 6.94 (d, $J = 8.6$ Hz, 1H), 6.86 (s, 1H), 5.69 (ddd, $J = 22.6$ Hz, 10.2 Hz & 5.6 Hz, 1H), 5.05 (d, $J = 18.3$ Hz, 1H), 5.01 (d, $J = 10.2$ Hz, 1H), 4.13 (dt, $J = 9.3$ Hz & 4.9 Hz, 1H), 3.69 (d, $J = 5.4$ Hz, 1H), 1.98 (d, $J = 5.4$ Hz, 1H), 1.32 (qd, $J = 13.3$ Hz & 4.5 Hz, 1H), 1.21 - 1.09 (m, 1H), 0.82 - 0.77 (m, 1H), 0.66 (d, $J = 6.8$ Hz, 3H), 0.51 (d, $J = 6.4$ Hz, 3H)

Synthesis of aziridine containing peptide (26b)

To a solution of the azido alcohol **25b** (309 mg, 0.9 mmol) in CH_3CN (7.2 mL) was added triphenylphosphine (236 mg, 0.9 mmol) in portions, with vigorous stirring at ambient conditions. The reaction mixture was stirred for a further 0.5 h at ambient temperature after complete addition of PPh_3 ; after which, the solution was set to reflux for 6 h. The solvent was evaporated under vacuum and the resulting residue was

subjected to flash column chromatography (Silica gel - EtOAc:Hexane - 3:2) to yield the corresponding aziridine containing peptide in moderate yields (54%).

^1H NMR, 400 MHz, CDCl_3 , δ 7.58 (dd, $J = 11.9$ Hz & 1.5 Hz, 1H, aziridine NH), 7.39 (dt, $J = 8.1$ Hz & 3.1 Hz, 0.4H, amide NH), 7.27 - 7.17 (m, 5H), 7.02 (bs, 0.6H, amide NH), 5.77 (ddd, $J = 22.5$ Hz, 10.5 Hz & 5.1 Hz, 1H), 5.10 (dd, $J = 15.9$ Hz & 1.4 Hz, 1H), 5.07 (dd, $J = 10.5$ Hz & 1.4 Hz, 1H), 4.53 (d, $J = 6.6$ Hz, 1H), 3.80 (dt, $J = 3.4$ Hz & 1.7 Hz, 2H), 3.57 (d, $J = 9.2$ Hz, 1H), 3.56 (dd, $J = 8.8$ Hz & 2.8 Hz, 1H), 3.07 (d, $J = 6.8$ Hz, 1H), 2.57 (d, $J = 6.8$ Hz, 1H), 2.37 (tdd, $J = 12.2$ Hz, 6.6 Hz & 2 Hz, 1H), 2.19 - 2.08 (m, 1H), 1.99 - 1.90 (m, 1H), 1.87 - 1.77 (m, 1H); MS m/z 300 (M^+), 283, 267, 243, 228, 210, 182, 155, 137

Synthesis of aziridine containing peptide (26c)

To a solution of the azido alcohol **25c** (912 mg, 2 mmol) in CH_3CN (16 mL) was added triphenylphosphine (526 mg, 2 mmol) in portions, with vigorous stirring at ambient conditions. The reaction mixture was stirred for a further 0.5 h at ambient temperature after complete addition of PPh_3 ; after which, the solution was set to reflux for 6 h. The solvent was evaporated under vacuum and the resulting residue was subjected to flash column chromatography (Silica gel - EtOAc:Hexane- 7:3) to yield the corresponding aziridine containing peptide in good yields (68%) $[\alpha]_{\text{D}}^{25} = -94^\circ$ ($c = 0.005$, CH_2Cl_2).

^1H NMR, 400 MHz, CDCl_3 , δ 7.31 - 7.19 (m, 5H), 7.13 (d, $J = 7.3$ Hz, 0.5H), 6.92 (d, $J = 5.9$ Hz, 0.5 H), 6.65 & 6.53 (m, 1H), 5.75 (ddd, $J = 20.5$ Hz, 11.2 Hz & 5.6 Hz, 1H), 5.11 (ddd, $J = 18.2$ Hz, 3.2 Hz & 1.7 Hz, 1H), 5.06 (td, $J = 10.2$ Hz & 1.2 Hz, 1H), 4.50 (dt, $J = 9.1$ Hz & 3.2 Hz, 1H), 4.34 (dd, $J = 8.32$ Hz & 4.9 Hz, 1H), 3.80 - 3.77 (m, 2H), 3.60 (dd, $J = 8.6$ Hz & 5.1 Hz, 1H), 3.51 (t, $J = 7.6$ Hz, 1H), 3.10, 3.04 (d, $J = 2.2$ Hz, 1H), 2.61 - 2.59 (d, $J = 2.2$ Hz, 1H), 2.26 - 2.20 (m, 1H), 2.18 - 2.14 (m, 1H), 2.08 - 1.97 (m, 2H), 1.73 - 1.67 (m, 1H), 1.52 (dt, $J = 17.8$ Hz & 5.6 Hz, 1H), 1.21 - 1.18 (m, 1H), 0.87 (dd, $J = 10.5$ Hz & 4.1 Hz, 3H), 0.84 (t, $J = 6.8$ Hz, 3H) MS m/z 413 (M^+), 391, 341, 307, 289, 268, 228, 209, 181, 154, 136.

Synthesis of aziridine containing peptide (26d)

To a solution of the azido alcohol **25d** (491 mg, 1 mmol) in CH_3CN (8 mL) was added triphenylphosphine (263 mg, 1 mmol) in portions, with vigorous stirring at ambient conditions. The reaction mixture was stirred for a further 0.5 h at ambient temperature after complete addition of PPh_3 ; after which, the solution was set to reflux for 6 h. The solvent was evaporated under vacuum and the resulting residue was subjected to flash column chromatography (Silica gel - EtOAc:Hexane- 7:3) to yield the corresponding aziridine containing peptide in good yields (65%) $[\alpha]_{\text{D}}^{25} = -79^\circ$ ($c = 0.0055$, CH_2Cl_2).

^1H NMR, 400 MHz, CDCl_3 , δ 7.40 - 7.26 (m, 5H), 7.24 - 7.11 (m, 5H), 6.94 (d, $J = 8.1$ Hz, 1H), 6.44 (t, $J = 5.6$ Hz, 1H), 5.75 (ddd, $J = 22.12$ Hz, 10.7 Hz & 5.6 Hz, 1H), 5.09 (dd, $J = 5.8$ Hz & 1.5 Hz, 1H), 5.07 (t, $J = 1.2$ Hz, 1H), 4.67 (dd, $J = 15.4$ Hz & 7.8 Hz, 1H), 4.48 (dd, $J = 7.8$ Hz & 3.2 Hz, 1H), 3.84 (dd, $J = 11.4$ Hz & 5.8 Hz, 2H), 3.58 (dd, $J = 9.3$ Hz & 6.6 Hz, 1H), 3.53 - 3.47 (m, 1H), 3.14 (d, $J = 6.8$ Hz, 2H), 3.11 & 3.01 (d, $J = 2.2$ Hz, 0.35 & 0.65 H), 2.59 & 2.52 (d, $J = 2.2$ Hz, 0.65 & 0.35 H), 2.19 - 2.10 (m, 2H), 1.95 - 1.85 (m, 2H); MS m/z 447 (M^+), 391, 302, 279, 243, 215, 200, 154, 136

Synthesis of aziridine containing peptide (26e)

To a solution of the azido alcohol **25e** (353 mg, 0.8 mL) in CH₃CN (6.4 mL) was added triphenylphosphine (210 mg, 0.8 mmol) in portions, with vigorous stirring at ambient conditions. The reaction mixture was stirred for a further 0.5 h at ambient temperature after complete addition of PPh₃; after which, the solution was set to reflux for 6 h. The solvent was evaporated under vacuum and the resulting residue was subjected to flash column chromatography (Silica gel - EtOAc:Hexane- 3:2) to yield the corresponding aziridine containing peptide in good yields (70%) [α]_D²⁵ = -139.7° (c = 0.018, CH₂Cl₂).

¹H NMR, 400 MHz, CDCl₃, δ 7.67 & 7.64 (d, J = 1.7 Hz, 1H), 7.47 (dd, J = 7.8 Hz & 2.9 Hz, 1H), 7.32 - 7.23 (m, 5H), 4.68 - 4.63 (m, 1H), 4.55 - 4.50 (m, 1H), 3.73 (s, 3H), 3.70 - 3.61 (m, 1H), 3.58 - 3.49 (m, 1H), 3.17 - 3.11 (d, J = 2.2 Hz, 1H), 2.66 (t, J = 2.4 Hz, 1H), 2.41 - 2.37 (m, 1H), 2.15 - 2.10 (m, 1H), 2.01 - 1.87 (m, 2H), 1.68 - 1.54 (m, 2H), 1.28 - 1.24 (m, 1H), 0.94 (dt, J = 10 Hz & 6 Hz, 6H)

General procedure for the cinnamoylation of Aziridine Containing Peptides

A solution of the aziridine containing peptide (1 equivalent) and triethylamine (1.1 equivalent) in THF (15 mL/mmol) was cooled to 0 °C in an ice bath. To it was added a solution of cinnamoyl chloride (1 equivalent) in THF (10 mL/mmol) drop wise at a slow rate (1 mL/min). The solution was warmed to room temperature after complete addition of cinnamoyl chloride and stirred for a further 6 h. The solvent was removed and the resulting residue was taken in EtOAc and washed with water and brine. The organic phase was separated, dried (Na₂SO₄) and concentrated to give a residue which was subjected to flash column chromatography (Silica gel- EtOAc:Hexane) to yield the cinnamoylated CCP containing peptide in good yields.

Synthesis of CCP containing peptide (27b)

A solution of the aziridine containing peptide **26b** (119 mg, 0.4 mmol) and triethylamine (0.06 mL) in THF (6 mL) was cooled to 0 °C in an ice bath. To it was added a solution of cinnamoyl chloride (67 mg, 0.4 mmol) in THF (4 mL) drop wise at a slow rate (1 mL/min). The solution was warmed to room temperature after complete addition of cinnamoyl chloride and stirred for a further 6 h. The solvent was removed and the resulting residue was taken in EtOAc (15 mL) and washed with water (2X5 mL) and brine (1X5 mL). The organic phase was separated, dried (Na₂SO₄) and concentrated to give a residue which was subjected to flash column chromatography (Silica gel- EtOAc:Hexane- 3:2) to yield the cinnamoylated CCP containing peptide in good yields (72%) [α]_D²⁵ = +32° (c = 0.005, CH₂Cl₂).

¹H NMR, 400 MHz, CDCl₃, δ 8.00 (bs, 1H), 7.52 (d, J = 16.1 Hz, 1H), 7.42 - 7.19 (m, 10H), 6.51 (d, J = 16.1 Hz, 1H), 5.83 - 5.70 (m, 1H), 5.15 (ddd, J = 9.3 Hz, 7.6 Hz & 1.7 Hz, 1H), 5.01 (ddd, J = 11.7 Hz, 8.8 Hz & 1.5 Hz, 1H), 4.57 (d, J = 7.8 Hz, 1H), 4.27 (d, J = 7.1 Hz, 1H), 3.87 - 3.82 (m, 1H), 3.62 - 3.57 (m, 1H), 2.39 (d, J = 7.8 Hz, 1H), 2.22 - 2.24 (m, 1H), 2.03 - 1.77 (m, 2H), 1.81 - 1.72 (m, 1H); MS *m/z* 430 (M⁺), 298, 194, 152

Synthesis of CCP containing peptide (27c)

A solution of the aziridine containing peptide **26c** (412 mg, 1 mmol) and triethylamine (1.4 mL) in THF (15 mL) was cooled to 0 °C in an ice bath. To it was added a solution of cinnamoyl chloride (167 mg, 1 mmol) in THF (10 mL) drop wise at a slow rate (1 mL/min). The solution was warmed to room temperature after complete addition of

cinnamoyl chloride and stirred for a further 6 h. The solvent was removed and the resulting residue was taken in EtOAc (25 mL) and washed with saturated solution of NaHCO₃ (2X10 mL) water (1X10 mL) and brine (2X10 mL). The organic phase was separated, dried (Na₂SO₄) and concentrated to give a residue which was subjected to flash column chromatography (Silica gel- EtOAc:Hexane- 7:3) to yield the cinnamoylated CCP containing peptide in good yields(75%) [α]_D²⁵ = +76 ° (c = 0.0015, CH₂Cl₂).

¹H NMR, 400 MHz, CDCl₃, δ 7.50 (d, J = 15.9 Hz, 1H), 7.46 - 7.24 (m, 10 H), 6.89 (t, J = 5.12 Hz, 1H), 6.54 (d, J = 15.9 Hz, 1H), 5.65 (ddd, J = 22.7 Hz, 11 Hz & 5.6 Hz, 1H), 4.97 (dd, J = 15.6 Hz & 1.7 Hz, 1H), 4.87 (dd, J = 10.2 Hz, 1.5 Hz, 1H), 4.55 (dd, J = 8.8 Hz & 2.4 Hz, 2H), 4.27 (d, J = 2.2 Hz, 1H), 4.18 (t, J = 7.4 Hz, 1H), 3.76 (dd, J = 9.8 Hz & 2.9 Hz, 2H), 3.48 (td, J = 15.6 Hz & 5.4 Hz, 1H), 3.25 (d, J = 2.2 Hz, 1H), 2.31 - 2.25 (m, 1H), 2.23 - 2.16 (m, 1H), 2.02 - 1.93 (m, 2H), 1.73 - 1.71 (m, 2H), 1.25 - 1.22 (m, 1H), 1.00 (d, J = 6.6 Hz, 3H), 0.94 (d, J = 6.6 Hz, 3H); MS *m/z* 543 (M⁺), 503, 486, 459, 414, 398, 373, 307, 268, 228, 200, 181, 154, 136

Synthesis of CCP containing peptide (27d)

A solution of the aziridine containing peptide **26d**(223 mg, 0.5 mmol) and triethylamine (0.08 mL) in THF (7.5 mL) was cooled to 0 °C in an ice bath. To it was added a solution of cinnamoyl chloride (85 mg, 0.5 mmol) in THF (5 mL) drop wise at a slow rate (1 mL/min). The solution was warmed to room temperature after complete addition of cinnamoyl chloride and stirred for a further 6 h. The solvent was removed and the resulting residue was taken in EtOAc (20 mL) and washed with saturated solution of NaHCO₃ (2X10 mL) water (2X10 mL) and brine (1X10 mL). The organic phase was separated, dried Na₂SO₄ and concentrated to give a residue which was subjected to flash column chromatography (Silica gel- EtOAc:Hexane- 7:3) to yield the cinnamoylated CCP containing peptide in good yields (75%) [α]_D²⁵ = +57 ° (c = 0.0015, CH₂Cl₂).

¹H NMR 400 MHz, CDCl₃ δ 7.64 (d, J = 15.4 Hz, 1H), 7.54 (d, J = 7.8 Hz, 1h), 7.42 - 7.38 (m, 2H), 7.34 - 7.22 (m, 6H), 7.18 - 7.11 (m, 2H), 6.79 (t, J = 5.6 Hz, 1H), 6.72 (d, J = 15.4 Hz, 1H), 5.76 - 5.58 (m, 1H), 5.51 (d, J = 1.7 Hz, 1H), 5.49 (d, J = 2.2 Hz, 1H), 5.00 (dd, J = 12.6 Hz & 6.8 Hz, 1H), 4.59 (d, J = 6.8 Hz, 1H), 4.26 (t, J = 6.8 Hz, 1H), 3.84 (dd, J = 5.12 Hz & 3.6 Hz, 2H), 3.77 (t, J = 5.4 Hz, 1H), 3.39 - 3.32 (m, 1H), 3.16 - 3.02 (m, 1H), 2.40 (dd, J = 6.8 Hz & 3.2 Hz, 2H), 2.24 - 2.11 (m, 1H), 1.99 - 1.84 (m, 2H)

Synthesis of CCP containing peptide (27e)

A solution of the aziridine containing peptide **26e** (119 mg, 0.3 mmol) and triethylamine (0.04 mL) in THF (5 mL) was cooled to 0 °C in an ice bath. To it was added a solution of cinnamoyl chloride (50 mg, 0.3 mmol) in THF (3 mL) drop wise at a slow rate (1 mL/min). The solution was warmed to room temperature after complete addition of cinnamoyl chloride and stirred for a further 6 h. The solvent was removed and the resulting residue was taken in EtOAc (10 mL) and washed with saturated solution of NaHCO₃ (2X5 mL) water (1X5 mL) and brine (2X5 mL).. The organic phase was separated, dried (Na₂SO₄) and concentrated to give a residue which was subjected to flash column chromatography (Silica gel- EtOAc:Hexane- 5:4) to yield the cinnamoylated CCP containing peptide in good yields (56%) [α]_D²⁵ = +12 ° (c = 0.002, CH₂Cl₂).

¹H NMR, 400 MHz, CDCl₃, δ 7.77 (d, J = 16 Hz, 1H), 7.66 - 7.58 (m, 3H), 7.48 - 7.21 (m, 7H), 6.77 (d, J = 8.2 Hz, 1H), 6.38 (d, J = 16 Hz, 1H), 5.21 (t, J = 3.2 Hz, 1H), 4.76

(d, $J = 5$ Hz, 1H), 4.48 (dd, $J = 7.1$ Hz & 3 Hz, 1H), 3.64 (s, 3H), 3.56 (dd, $J = 22.9$ Hz & 9.5 Hz, 2H), 2.26 - 2.12 (m, 1H), 2.10 - 1.88 (m, 3H), 1.73 - 1.50 (m, 2H), 1.21 - 1.12 (m, 1H), 0.84 (d, $J = 6.6$ Hz, 3H), 0.81 (d, $J = 6.6$ Hz, 3H)

Synthesis of compound C

A solution of the aziridine containing peptide **26b** (90 mg, 0.3 mmol) and triethylamine (0.04 mL) in THF (2 mL) was cooled to 0 °C in an ice bath. To it was added a solution of cinnamoyl chloride (50 mg, 0.3 mmol) in THF (2 mL). The solution was warmed to room temperature after complete addition of cinnamoyl chloride and stirred for a further 6 h. The solvent was removed and the resulting residue was taken in EtOAc (10 mL/mmol) and washed with saturated solution of NaHCO₃ (2X3 mL) water (2X3 mL) and brine (2X3 mL). The organic phase was separated, dried (Na₂SO₄) and concentrated to give a residue which was subjected to flash column chromatography (Silica gel-EtOAc:Hexane- 3:2) to yield compound **C** as the major product in good yields (60%) as a solid (M.P = 63°C).

¹H NMR, 400 MHz, CDCl₃, δ 7.66 (ddd, $J = 12$ Hz, 8.3 Hz & 5.5 Hz, 1H), 7.56 - 7.44 (m, 4H), 7.53 (d, $J = 16.1$ Hz, 1H), 6.20 & 6.14 (d, $J = 7.3$ Hz, 0.4H & 0.6 H), 5.90 - 5.76 (m, 1H), 5.18 (ddd, $J = 17.1$ Hz, 1.7 Hz & 1.5 Hz, 1H), 5.11 (td, $J = 10.2$ Hz & 1.5 Hz, 1H), 4.80 & 4.74 (d, $J = 7.3$ Hz, 0.6H & 0.4H), 4.63 - 4.59 (m, 1H), 4.04 - 3.99 (m, 1H), 3.80 (dt, $J = 5.6$ Hz & 1.5 Hz, 2H), 3.57 (dd, $J = 10$ Hz & 3.7 Hz, 1H), 2.39 - 2.36 (m, 1H), 2.16 - 2.08 (m, 1H), 2.04 - 1.94 (m, 2H); MS m/z 430 (M⁺), 345, 307, 279, 181, 154

Synthesis of CCP containing pentapeptide (28)

A solution of N-cinnamoyl-leucine (35 mg, 0.13 mmol) in dichloromethane (10 mL) was cooled to 0 °C in a ice-bath and triethylamine (0.02 mL, 0.12 mmol) was added to it while stirring, followed by HOBt (18 mg, 0.13 mmol). The reaction mixture was stirred for 10 min and the aziridine containing peptide (50 mg, 0.12 mmol) was added, followed by stirring at 0 °C for 10 min. Then DCC (28 mg, 1.3 mmol) and triethylamine (0.02 mL, 0.12 mmol, 1.1 equivalents) were added. The solution was warmed to room temperature and stirred for a further 18 h. The reaction mixture was diluted with DCM (10 mL) and the organic layer was washed with saturated solution of NaHCO₃ (2X3 mL), water (2X3 mL) and brine (1X3 mL). The organic layer was separated, dried (Na₂SO₄) and concentrated in vacuo to yield a residue which was subjected to flash column chromatography (Silica gel - EtOAc:hexane - 4:1) to yield the pentapeptide **28** as a solid (M.P. - 163 -165 °C) in good yields (61 %), $[\alpha]_D^{25} = -12^\circ$ (c = 0.001, CH₂Cl₂).

¹H NMR, 400 MHz, CDCl₃ δ 7.81 (d, $J = 8.2$ Hz, 1H), 7.50 (d, $J = 15.4$ Hz, 1H), 7.41 - 7.23 (m, 5H), 7.19 - 6.99 (m, 5H), 6.43 (d, $J = 8.7$ Hz, 1H), 6.28 (d, $J = 15.4$ Hz, 1H), 5.97 (t, $J = 5.8$ Hz, 1H), 5.90 (d, $J = 10.7$ Hz, 1H), 5.87 - 5.76 (m, 1H), 5.17 (d, $J = 18.3$ Hz, 1H), 5.06 (d, $J = 9.5$ Hz, 1H), 4.59 (d, $J = 10.7$ Hz, 1H), 4.46 (m, 1H), 4.37 (dt, $J = 5.6$ Hz & 1.4 Hz, 1H), 4.24 (dd, $J = 4.8$ Hz & 2.1 Hz, 1H), 4.24 (dd, $J = 4.8$ Hz & 2.1 Hz, 1H), 4.08 (dd, $J = 6.1$ Hz & 2.4 Hz, 1H), 4.00 - 3.93 (m, 1H), 3.83 - 3.78 (m, 1H), 3.76 - 3.72 (m, 1H), 2.26 - 2.20 (m, 1H), 2.16 - 1.90 (m, 2H), 1.73 (bs, 1H), 1.20 - 1.16 (m, 2H), 0.93 - 0.89 (m, 1H), 0.81 (t, $J = 7.3$ Hz, 6H), 0.67 (d, $J = 6.4$ Hz, 6H); MS m/z 673(M⁺), 634, 613, 543, 482, 460, 443, 392, 329, 307, 289, 273, 242, 154, 136

Synthesis of azido alcohol (30)

To a solution of ethyl-3-phenyl glycidate (192 mg, 1 mmol) in methanol (8 mL) was added NaN₃ (195 mg, 3 mmol) and NH₄Cl (118 mg, 2.2 mmol) followed by water (1 mL)

and the reaction mixture was stirred at ambient conditions for 18 h. The solvent was removed to give a residue which was taken in EtOAc (15 mL) and washed with water (2X5 mL) and the organic layer was dried (Na_2SO_4) and concentrated in vacuum to yield a residue which was purified by subjection to column chromatography (Silica gel-EtOAc:hexane- 1:7) to yield the corresponding azido alcohol in good yields(68%).

^1H NMR, 80 MHz, CDCl_3 , δ 7.58 (s, 5H), 5.19 (d, J = 5.6 Hz, 1H), 4.94 (d, J = 4.6 Hz, 1H), 3.82 (q, J = 4.5 Hz, 2H), 2.01 (t, J = 5.6 Hz, 3H); IR ν_{max} 3300 - 3200 (br), 2910, 2880, 1750, 1660

Synthesis of conformationally constrained phenylalaninate (**31**)

To a solution of the azido alcohol **30** (117 mg, 0.5 mmol) in CH_3CN (4 mL) was added triphenylphosphine (131 mg, 0.5 mmol) in portions, with vigorous stirring at ambient conditions. The reaction mixture was stirred for a further 0.5 h at ambient temperature after complete addition of PPh_3 , after which, the solution was set to reflux for 6 h. The solvent was evaporated under vacuum and the resulting residue was subjected to flash column chromatography (Silica gel - EtOAc:Hexane- 1:32) to yield conformationally constrained phenylalaninate in good yields (66%).

^1H NMR, 80 MHz, CDCl_3 , δ 7.58 (s, 5H), 3.89 (q, J = 5.6 Hz, 2H), 3.12 (d, J = 4.1 Hz, 1H), 2.21 (d, J = 4.1 Hz, 1H), 2.01 (t, J = 5.6 Hz, 3H)

Synthesis of CCP containing peptide (**32**)

A stirring solution of N-cinnamoyl-phenylalanine-proline (110 mg, 0.28 mmol) and triethylamine (0.04 mL, 0.28 mmol) in THF (10 mL) was cooled to -10°C in an ice-salt bath and to it was added isobutylchloroformate (0.036 mL, 0.28 mmol) and stirred vigorously for 50 - 60 seconds. Then a solution of **31** (48 mg, 0.25 mmol) in THF (2 mL) was added followed by a solution of triethylamine (0.04 mL, 0.28 mmol) in THF (10 mL). The mixture was warmed to room temperature by removal of the ice bath and vigorously stirred for further 4 h. Triethylamine hydrochloride was filtered off on a sintered funnel under suction and washed twice with THF. Removal of solvent from the filtrate under vacuo yielded a residue, which was dissolved in EtOAc (10 mL) and washed with saturated aqueous solution of NaHCO_3 (2X2 mL), water (2X2 mL) and brine (1X2 mL). Drying (Na_2SO_4) and evaporation of solvent under vacuo yielded the crude product which was further purified by flash column chromatography (EtOAc:Hexane-3:2) (TLC - R_f = 0.5; hexane:ethylacetate 7:3) to yield the CCP containing peptide **32** as a gum in good yields (61 %) $[\alpha]_{\text{D}}^{25} = -0.8^\circ$ (c = 0.001, CH_2Cl_2).

^1H NMR, 400 MHz, CDCl_3 , δ 7.56 (d, J = 15.84 Hz, 1H), 7.54 - 7.50 (m, 1H), 7.47 - 7.45 (m, 2H), 7.41 - 7.29 (m, 7H), 7.26 - 7.17 (m, 5 H), 6.52 (d, J = 8.8 Hz, 1H), 6.35 (d, J = 15.84 Hz, 1H), 5.09 (dd, J = 13.4 Hz & 7.3 Hz, 1H), 4.69 (dd, J = 7.8 Hz & 3.6 Hz, 1H), 4.30 (q, J = 7.32 Hz, 2H), 4.26 (d, J = 2.4 Hz, 1H), 3.75 - 3.66 (m, 1H), 3.47 (dd, J = 8.3 Hz, 1H), 3.23 (d, J = 2.4 Hz, 1H), 3.16 (dd, J = 9.6 Hz & 7.6 Hz, 1H), 3.05 (dd, J = 9.9 Hz & 5.6 Hz, 1H), 2.17 - 2.14 (m, 1H), 2.09 - 2.04 (m, 2H), 1.95 - 1.91 (m, 1H); IR ν_{max} 3300, 2980, 1775, 1675, 1660, 1610, 1438 cm^{-1}

Synthesis of methyl-N-cinnamoyl-L-phenylalaninate

To a stirring ice cold solution of cinnamoyl chloride (1.65 gm, 10 mmol) in dichloromethane (15 mL) was added methylphenylalaninate hydrochloride (2.37 gm, 11 mmol) followed by a solution of triethylamine (3.1 mL, 22 mmol) in dichloromethane

(15 mL) drop wise through a dropping funnel. After complete addition of triethylamine, the reaction mixture was vigorously stirred for a further 5-6 h and then diluted with dichloromethane (15 mL). The organic layer was washed with saturated aqueous solution of NaHCO_3 , water and brine. Drying (Na_2SO_4) and evaporation of solvent under vacuo yielded the crude product which was further purified by column chromatography (EtOAc:Hexane = 1:5) (TLC - R_f = 0.5; hexane ethylacetate 4:1) to yield the required product as a crystalline white solid (M.P. = 78 °C) in good yields (80 %).

^1H NMR, 80 MHz, CDCl_3 , δ 7.62 (d, J = 16 Hz, 1H), 7.38 - 7.15 (m, 10H), 6.53 (d, J = 16 Hz, 1H), 5.08 (dd, J = 13.5 Hz & J = 6 Hz, 1H), 3.85 (s, 3H), 3.22 (d, J = 8.2 Hz, 2H)

Synthesis of N-cinnamoyl-phenylalanine

To a stirring ice cold solution of triethylamine (3.1 mL, 22 mmol) in acetonitrile (30 mL) was added L-phenylalanine (1.65 gm, 10 mmol). A solution of cinnamoyl chloride (1.67 gm, 10 mmol) in acetonitrile (15 mL) was added drop wise to this solution at such a rate that the reaction vessel temperature did not exceed 10 °C. After complete addition, the reaction mixture was warmed to room temperature and stirring was continued for 4-5 h. Following acidification and isolation as described in method A yielded N-cinnamoyl-L-phenylalanine as a sticky white solid in good yields (85 %).

Synthesis of methyl-N-cinnamoyl-L-phenylalanine-L-prolinate

A stirring solution of N-cinnamoyl-L-phenylalanine (1.33 gm, 4.5 mmol) and triethylamine (0.63 mL, 4.5 mmol) in THF (10 mL) was cooled to -10 °C in an ice-salt bath and to it was added methylchloroformate (0.35 mL, 4.5 mmol) and stirred vigorously for 50 - 60 seconds. Then a solution of methyl-4-trans-hydroxy-L-prolinate hydrochloride (998 mg, 4.95 mmol) in DMSO (2.5 mL) was added followed by a solution of triethylamine (1.4 mL, 9.9 mmol) in THF (15 mL). The mixture was warmed to room temperature by removal of the ice bath and vigorously stirred for further 4 h. Triethylamine hydrochloride was filtered off on a sintered funnel under suction. Removal of solvent from the filtrate under vacuo yielded a residue, which was dissolved in EtOAc (25 mL) and washed with saturated aqueous solution of NaHCO_3 (2X10 mL), water (2X10 mL) and brine (1X10 mL). Drying (Na_2SO_4) and evaporation of solvent under vacuo yielded the crude product which was further purified by column chromatography (EtOAc:Hexane-1:1) (TLC - R_f = 0.45; hexane : ethylacetate 1:1) to yield methyl-N-cinnamoyl-phenylalanine-prolinate as a gum, in good yields (67 %).

^1H NMR, 400 MHz, CDCl_3 , δ 7.80 (d, J = 8 Hz, 1H), 7.73 (d, J = 15.4 Hz, 1H), 7.57 - 7.51 (m, 2H), 7.43 - 7.39 (m, 3H), 7.19 - 7.10 (m, 5H), 6.67 (d, J = 15.4 Hz, 1H), 4.84 (dt, J = 8.1 Hz & 5.4 Hz, 1H), 4.73 (d, J = 7.6 Hz, 1H), 3.73 (s, 3H), 3.54 - 3.51 (m, 2H), 3.19 (dd, J = 13.9 Hz & 5.4 Hz, 1H), 2.96 (dd, J = 13.9 Hz & 8 Hz, 1H), 2.42 - 2.39 (m, 1H), 1.98 - 1.90 (m, 2H), 1.79 - 1.72 (m, 1H)

Synthesis of N-cinnamoyl-phenylalanine-proline

To a solution of the methyl-N-cinnamoyl-phenylalanine-prolinate (1.22 gm, 3 mmol) in MeOH (20 mL) was added a solution of $\text{LiOH}\cdot\text{H}_2\text{O}$ (123 mg, 3 mmol) in water (5 mL) and stirred at room temperature until completion of reaction (TLC - EtOAc : hexane - 1 : 1 - complete disappearance of starting material). Methanol was removed under vacuo and the aqueous part was acidified with an aqueous solution of 1N HCl by drop wise addition and simultaneous vigorous stirring. After complete acidification (no more milky white precipitate occurs on addition of dilute HCl), the resulting residue was extracted with

dichloromethane (3X10 mL), dried (anhydrous sodium sulphate) and concentrated under vacuo to yield N-cinnamoyl-phenylalanine-proline, in good yields (72 %) as a gum.

Synthesis of methyl-N-cinnamoyl-phenylalanine-proline-phenylalaninate (33)

A stirring solution of N-cinnamoyl-phenylalanine-proline (196 mg, 0.5 mmol) and triethylamine (0.07 mL, 0.5 mmol) in THF (2 mL) was cooled to -10 °C in an ice-salt bath and to it was added isobutylchloroformate (0.07 mL, 0.5 mmol) and stirred vigorously for 50 - 60 seconds. Then a solution of methyl-L-phenylalaninate hydrochloride (118 mg, 0.55 mmol) in DMSO (0.5 mL) was added followed by a solution of triethylamine (0.16 mL, 0.11 mmol) in THF (2 mL). The mixture was warmed to room temperature by removal of the ice bath and vigorously stirred for further 4 h. Triethylamine hydrochloride was filtered off on a sintered funnel under suction and washed twice with THF. Removal of solvent from the filtrate under vacuo yielded a residue, which was dissolved in EtOAc (10 mL) and washed with saturated aqueous solution of NaHCO₃ (2X3 mL), water (2X3 mL) and brine (1X3 mL). Drying (Na₂SO₄) and evaporation of solvent under vacuo yielded the crude product which was further purified by column chromatography (EtOAc:Hexane-3:2) (TLC - R_f = 0.4; hexane:ethylacetate 2:3) to yield methyl-N-cinnamoyl-phenylalanine-proline-phenylalaninate as a gum in good yields (77 %).

¹H NMR, 400 MHz, CDCl₃, δ 7.60 (d, J = 15.9 Hz, 1H), 7.50 - 7.48 (m, 2H), 7.40 (d, J = 8.3 Hz, 1H), 7.37 - 7.35 (m, 3H), 7.26 - 7.12 (m, 5H), 6.74 (d, J = 6.8 Hz, 1H), 6.46 (d, J = 15.9 Hz, 1H), 4.88 (dd, J = 11.6 Hz & 6.6 Hz, 1H), 4.80 (dt, J = 8.8 Hz & 5.4 Hz, 1H), 4.44 (d, J = 6.1 Hz, 1H), 3.66 (d, J = 4.4 Hz, 1H), 3.61 (s, 3H), 3.46 (dt, J = 7.8 Hz & 1.2 Hz, 1H), 3.22 (dd, J = 13.9 Hz & 5.4 Hz, 1H), 3.11 - 2.98 (m, 3H), 2.55 (dd, J = 16.8 Hz & 8.9 Hz, 1H), 2.09 - 2.04 (m, 2H), 1.49 - 1.47 (m, 1H); IR Data ν_{max} 3200, 2990, 2880, 1775, 1650, 1610

General Procedure for the Synthesis of β-substituted phenylalanine derivative containing peptides

To a solution of the N-cinnamoylated-CCP containing peptide (1 equivalent) in corresponding alcohol (10 mL/mmol) was added tosylic acid (catalytic) and the reaction mixture was stirred for 4 h. The solvent was removed and the resulting residue was taken in EtOAc and washed with saturated solution of NaHCO₃ and water. The organic phase was separated, dried (Na₂SO₄) and concentrated to yield a residue, which was subjected to flash column chromatography to yield the required product in good yields.

Synthesis of β-substituted phenylalanine containing peptide (34a)

To a solution of the N-cinnamoylated-CCP containing peptide 26c (60 mg, 0.146 mmol) in allyl alcohol (10 mL) was added tosylic acid (catalytic) and the reaction mixture was stirred for 4 h. The solvent was removed and the resulting residue was taken in EtOAc (10 mL) and washed with saturated solution of NaHCO₃ (2X3 mL) and water (2X3 mL). The organic phase was separated, dried (Na₂SO₄) and concentrated to yield a residue, which was subjected to flash column chromatography (Silicagel; EtOAc:hexane - 4:1) to yield the required product in good yields (71%) [α]_D²⁵ = -11 ° (c = 0.0005, CH₂Cl₂).

¹H NMR, 400 MHz, CDCl₃, δ 7.58 (s, 1H), 7.51 - 7.47 (m, 5H), 7.39 - 7.29 (m, 6H), 6.99 (d, J = 7.8 Hz, 1H), 5.87 - 5.78 (m, 2H), 4.13 (d, J = 17.6 Hz, 2H), 5.06 (d, J = 10.2 Hz, 1H), 5.00 (t, J = 8.8 Hz, 1H), 4.94 - 4.87 (m, 1H), 4.72 (d, J = 7.3 Hz, 1H), 4.55 - 4.51

(m, 1H), 4.38 (t, $J = 6.2$ Hz, 1H), 4.31 (d, $J = 4$ Hz, 1H), 4.06 - 4.00 (m, 1H), 3.97 - 3.93 (m, 1H), 3.83 - 3.80 (m, 2H), 3.67 (t, $J = 7.8$ Hz, 1H), 3.58 (t, $J = 5.4$ Hz, 1H), 3.49 - 3.45 (m, 1H), 2.00 - 1.98 (m, 1H), 1.82 - 1.63 (m, 3H), 1.53 (dt, $J = 12$ Hz & 5.1 Hz, 2H), 1.18 (s, 1H), 0.91 (dd, $J = 14.3$ Hz & 6.6 Hz, 3H), 0.83 (dd, $J = 6$ Hz & 5.4 Hz, 3H); MS m/z 601(M^+), 575, 560, 544, 307, 294, 266

Synthesis of β -substituted phenylalanine containing peptide (34b)

To a solution of the N-cinnamoylated-CCP containing peptide **26c** (60 mg, 0.146 mmol) in THF (10 mL) was added tosylic acid (catalytic) and water (0.5 mL) and the reaction mixture was stirred for 4 h. The solvent was removed and the resulting residue was taken in EtOAc (10 mL) and washed with saturated solution of NaHCO_3 (2X3 mL) and water (2X3 mL). The organic phase was separated, dried (Na_2SO_4) and concentrated to yield a residue, which was subjected to flash column chromatography (Silicagel; EtOAc:hexane - 1:3) to yield the required product in good yields (66%) $[\alpha]_D^{25} = -8^\circ$ ($c = 0.001$, CH_2Cl_2).

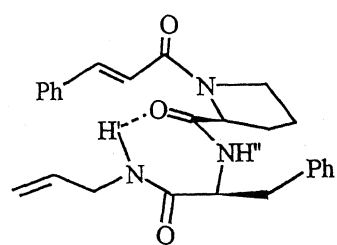
^1H NMR, 400 MHz, CDCl_3 δ 7.74 (d, $J = 15.6$ Hz, 1H), 7.47 (t, $J = 6.8$ Hz, 1H), 7.45 - 7.37 (m, 5H), 7.35 - 7.26 (m, 5H), 7.05 (d, $J = 7.9$ Hz, 1H), 5.84 (qd, $J = 22.7$ Hz & 5.6 Hz, 1H), 5.74 (d, $J = 6.1$ Hz, 1H), 5.18 (d, $J = 17.3$ Hz, 1H), 5.10 (t, $J = 8.8$ Hz, 1H), 5.04 (dd, $J = 4.8$ Hz & 1.8 Hz, 1H), 4.69 (dd, $J = 8$ Hz & 2.4 Hz, 1H), 4.39 (dd, $J = 17.8$ Hz & 9.6 Hz, 1H), 3.88 - 3.84 (m, 1H), 3.78 (td, $J = 17.36$ Hz & 3.7 Hz, 2H), 3.69 (dd, $J = 15.7$ Hz & 8.1 Hz, 1H), 2.32 - 2.27 (m, 1H), 2.12 - 2.05 (m, 1H), 2.01 (dd, $J = 16.6$ Hz & 9.3 Hz, 1H), 2.32 - 2.227 (m, 1H), 2.12 - 2.05 (m, 1H), 2.01 (dd, $J = 16.6$ Hz & 9.3 Hz, 1H), 1.83 (t, $J = 4.2$ Hz, 1H), 1.59 - 1.49 (m, 2H), 1.26 - 1.22 (m, 1H), 0.88 (dd, $J = 11.16$ Hz & 6.4 Hz, 6 H); MS m/z 561(M^+), 543, 504, 477, 455, 420, 398, 341, 307, 289, 268, 200, 188, 154, 131

Synthesis of β -substituted phenylalanine containing peptide (34c)

To a solution of the cinnamoylated-CCP containing peptide **26c** (60 mg, 0.146 mmol) in dry methanol (10 mL) was added tosylic acid (catalytic) and the reaction mixture was stirred for 4 h. The solvent was removed and the resulting residue was taken in EtOAc and washed with saturated solution of NaHCO_3 and water. The organic phase was separated, dried (Na_2SO_4) and concentrated to yield a residue, which was subjected to flash column chromatography (Silicagel; EtOAc:hexane - 4:1) to yield the required product in good yields (60%).

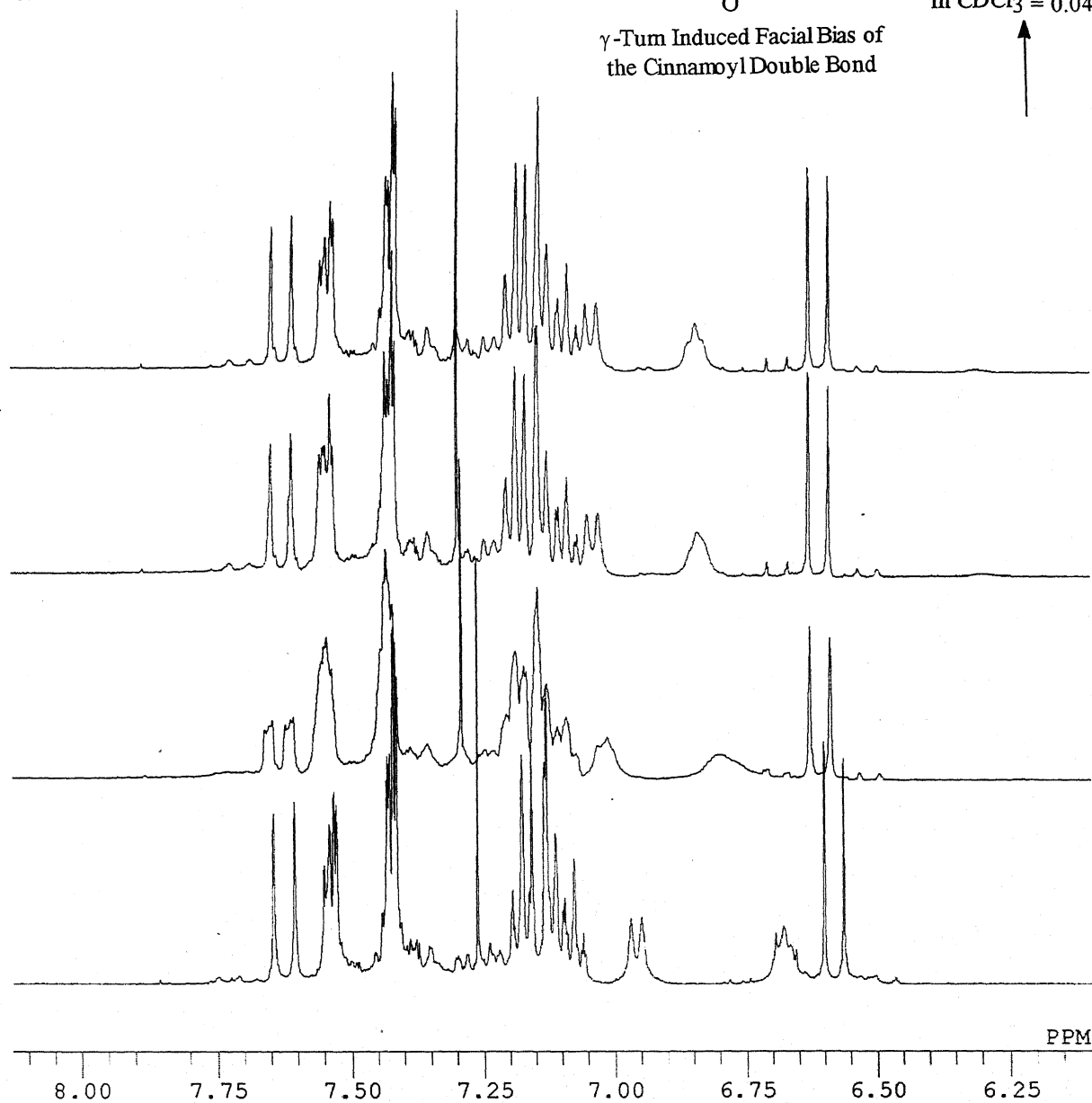
^1H NMR, 400 MHz, CDCl_3 , δ 7.74 (d, $J = 15.6$ Hz, 1H), 7.54 (dd, $J = 3.9$ Hz & 1.7 Hz, 1H), 7.44 - 7.37 (m, 6H), 7.33 (d, $J = 2.9$ Hz, 4H), 7.13 (d, $J = 7.8$ Hz, 1H), 5.84 (td, $J = 16.6$ Hz & 5.6 Hz, 1H), 5.22 (d, $J = 10.1$ Hz, 1H), 5.12 (d, $J = 9.1$ Hz, 1H), 4.70 (dd, $J = 8.8$ Hz & 2.4 Hz, 1H), 4.56 (dd, $J = 8.5$ Hz & 4.1 Hz, 1H), 4.41 (td, $J = 18$ Hz & 4.2 Hz, 2H), 3.91 - 3.87 (m, 2H), 3.81 - 3.74 (m, 1H), 3.70 (dd, $J = 16$ Hz & 9.2 Hz), 3.17 s, 3H), 2.37 - 2.33 (m, 1H), 2.21 - 1.82 (m, 3H), 1.61 - 1.44 (m, 2H), 1.25 (bs, 1H), 0.87 (dd, $J = 11.5$ Hz & 6.6 Hz, 6 H); MS m/z 575 (M^+), 543, 477, 455, 420, 398, 329, 307, 289, 268, 228, 200, 176, 154, 131

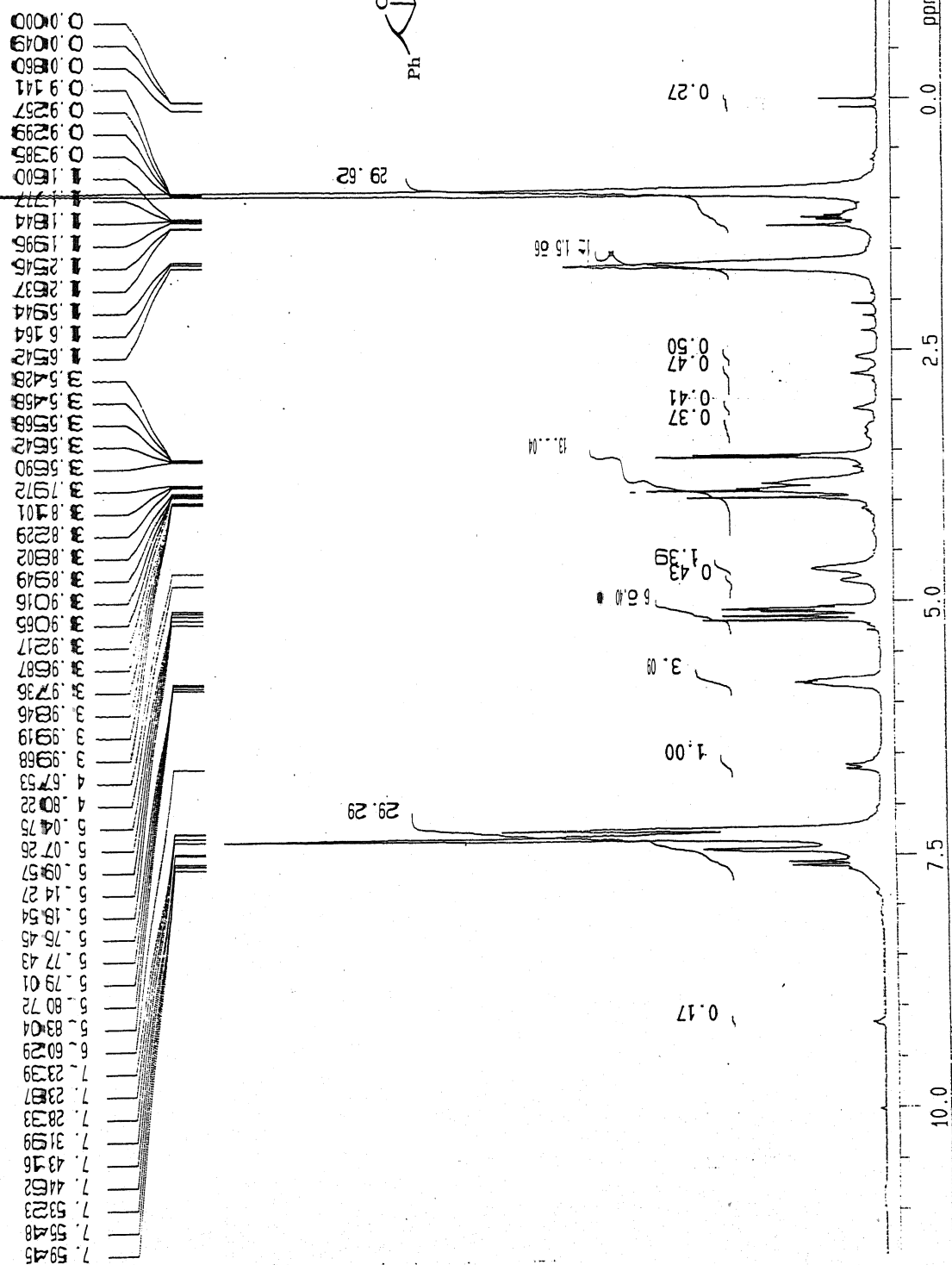
CPFM-1H

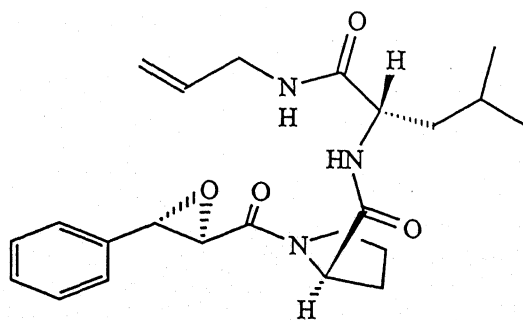
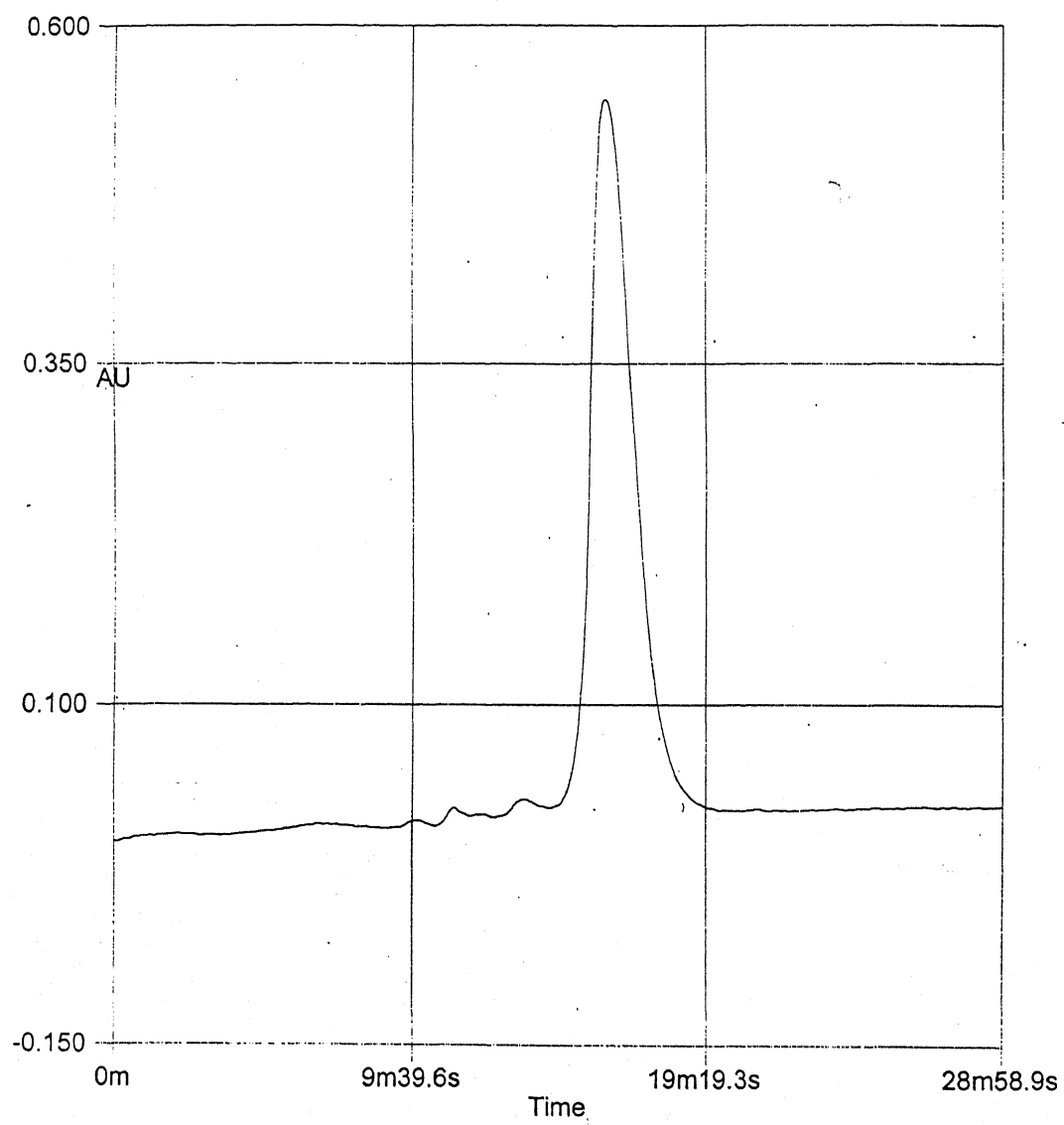


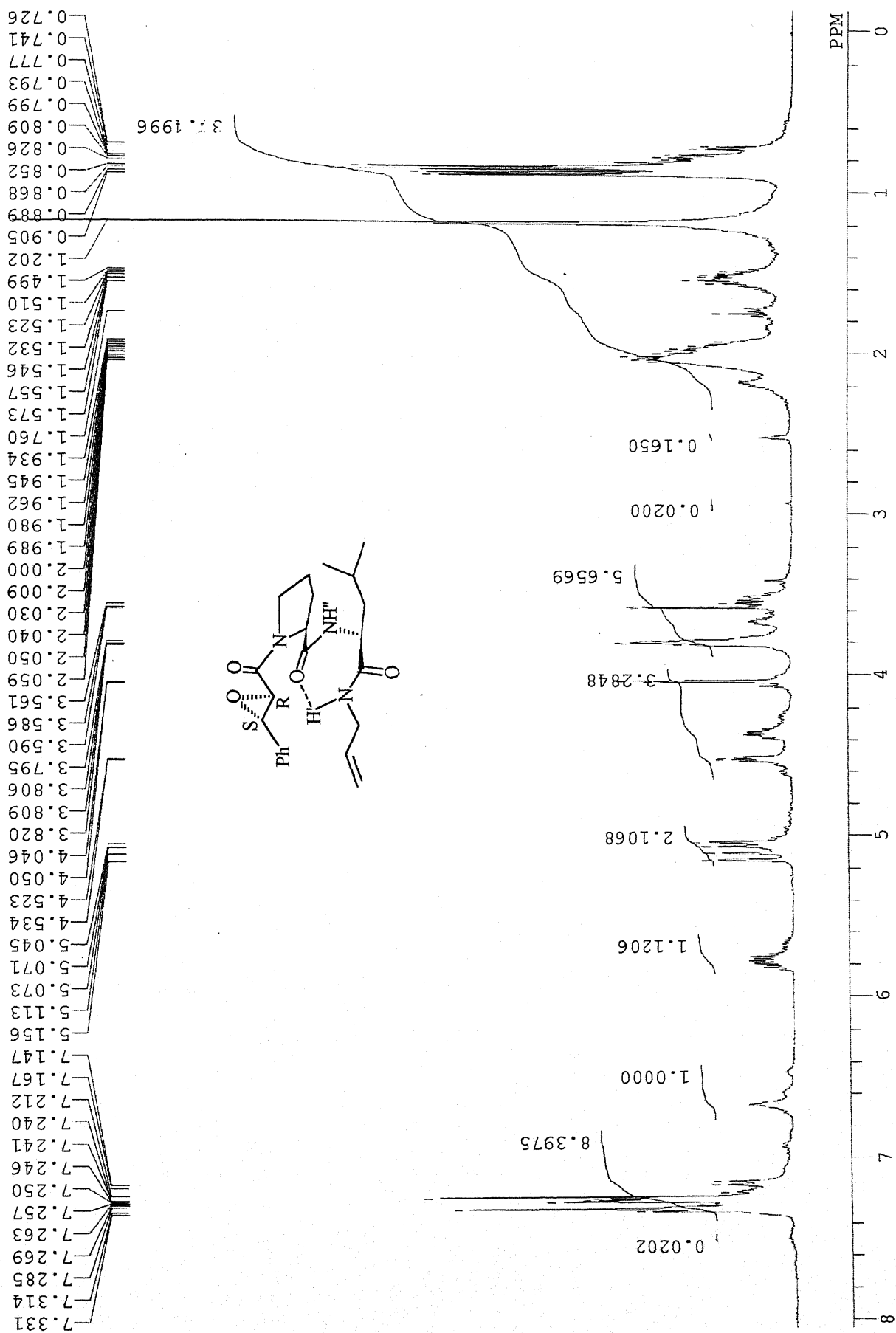
γ -Turn Induced Facial Bias of
the Cinnamoyl Double Bond

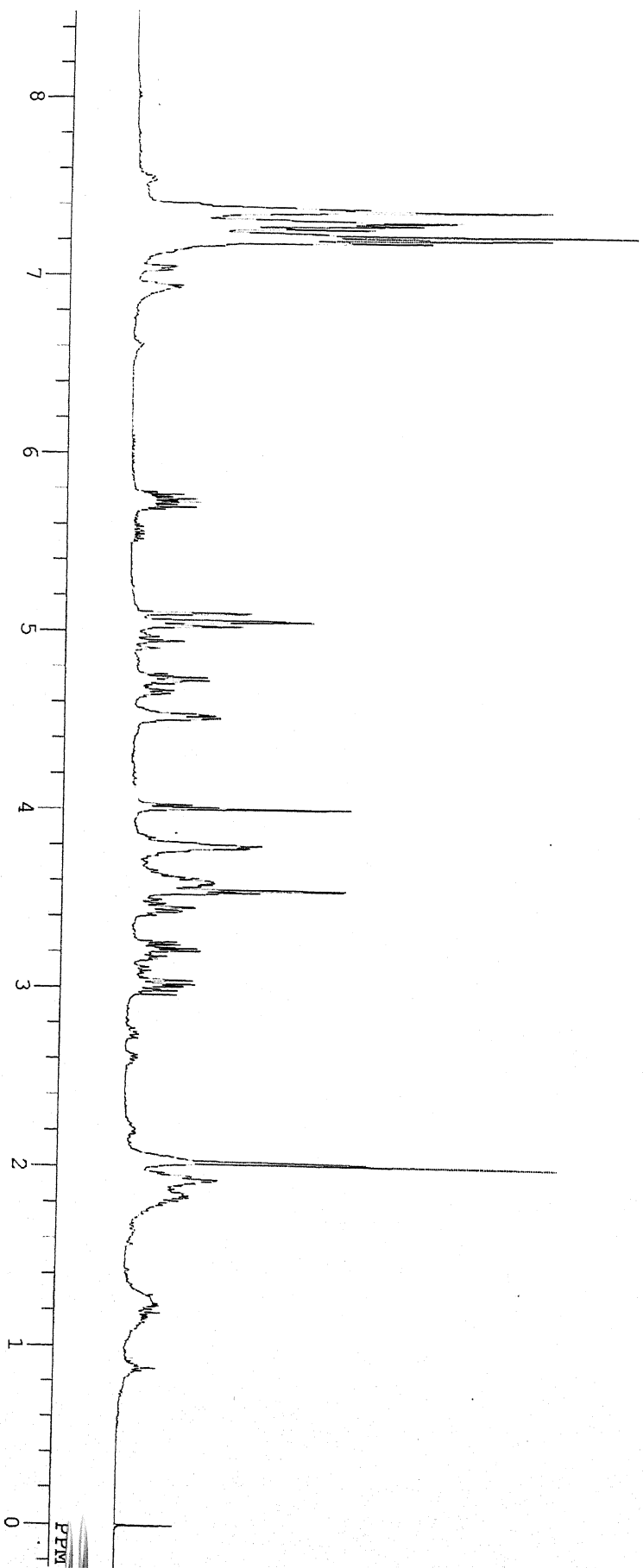
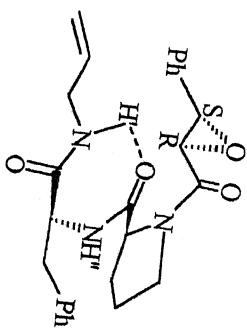
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in steps of 0.05 mL
Sample Concentration
in CDCl₃ = 0.04 mM

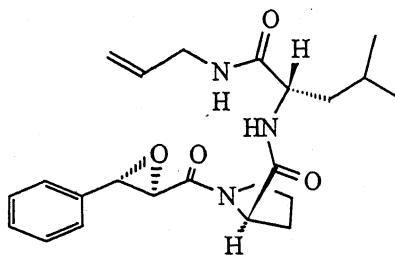




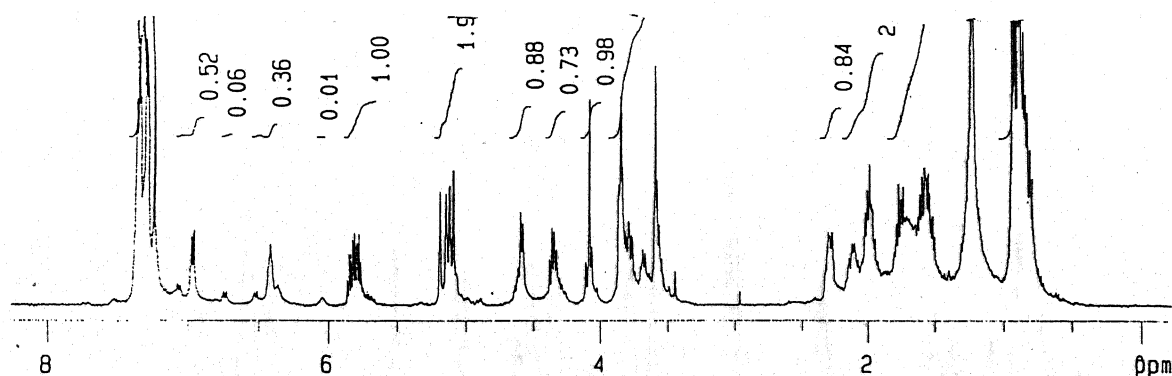
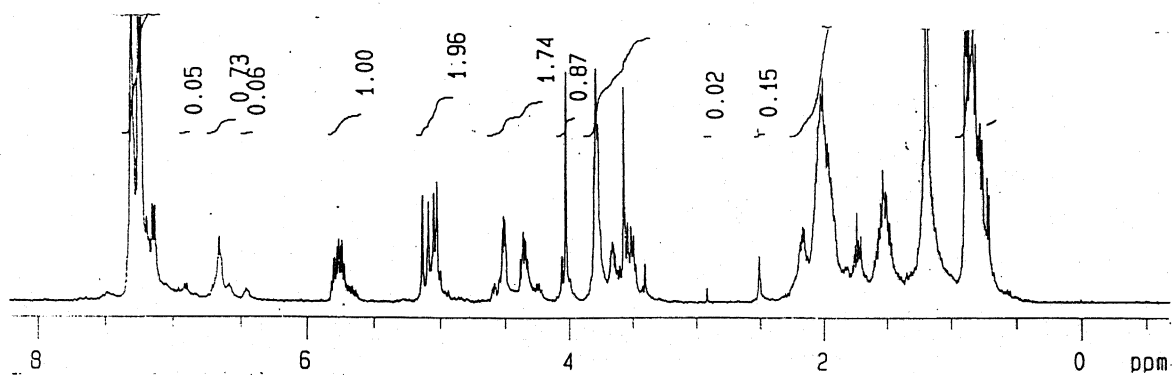
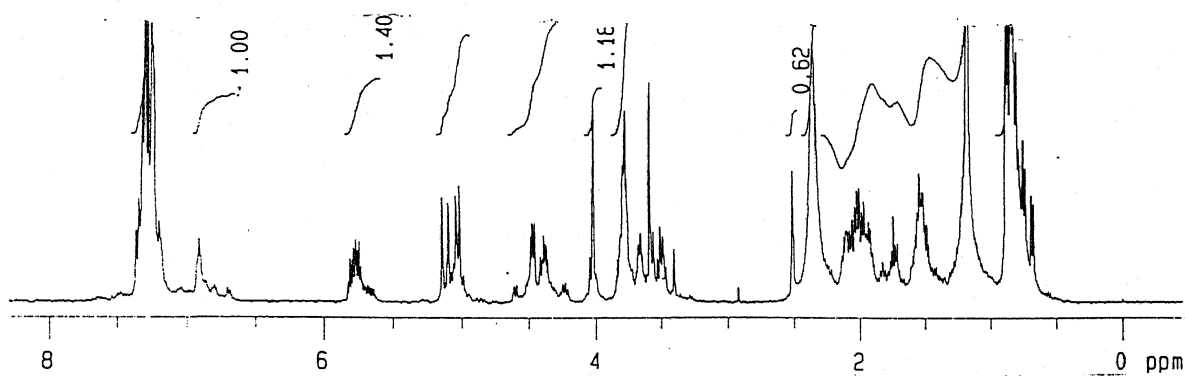
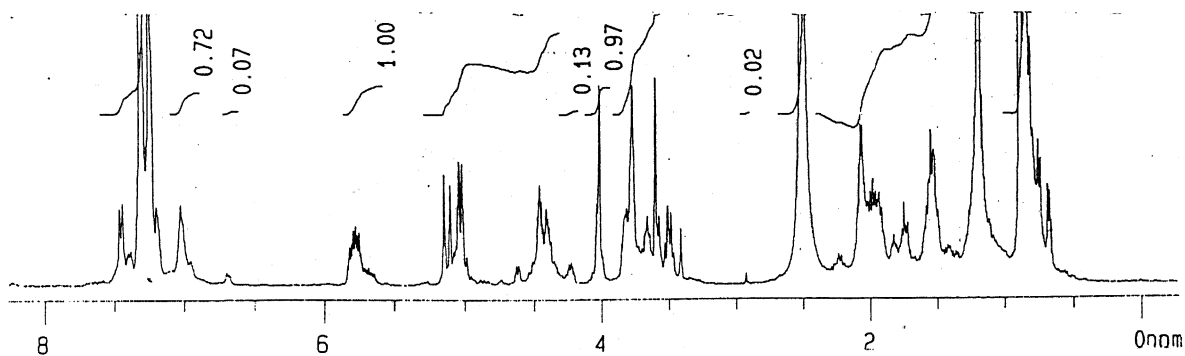


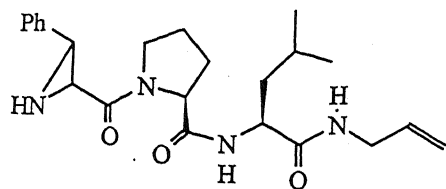




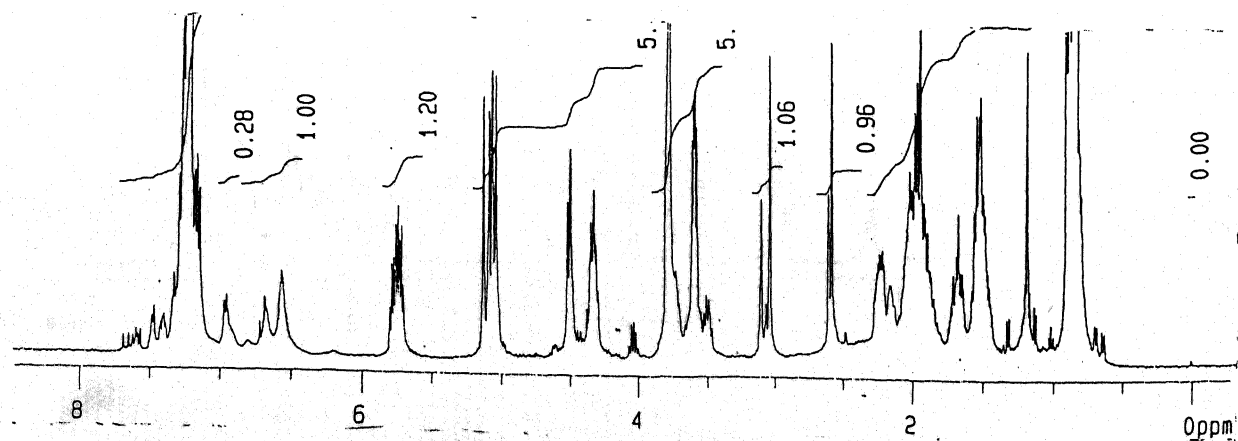
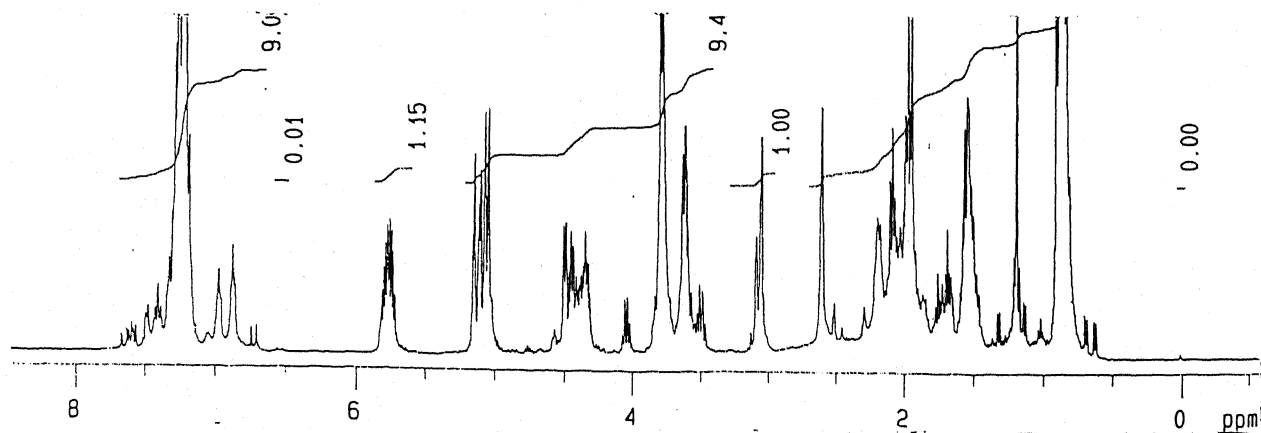
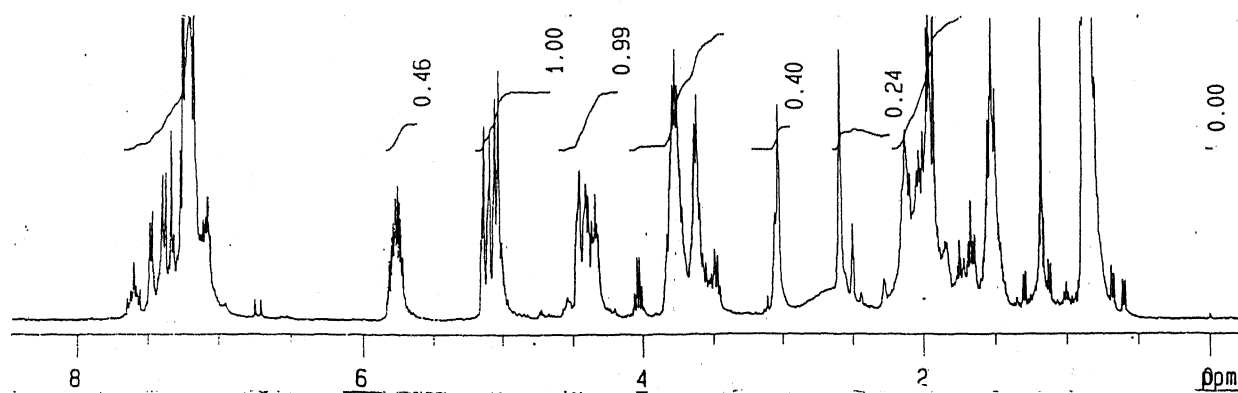
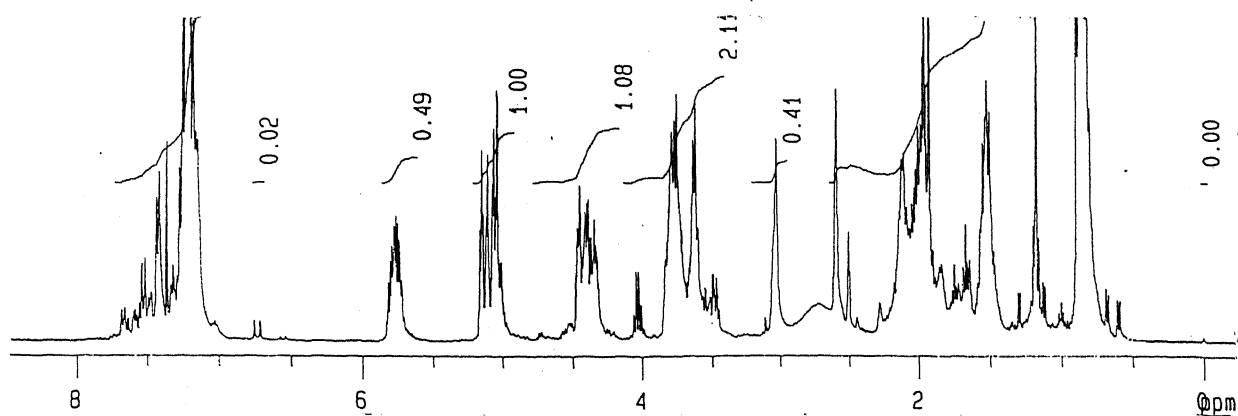


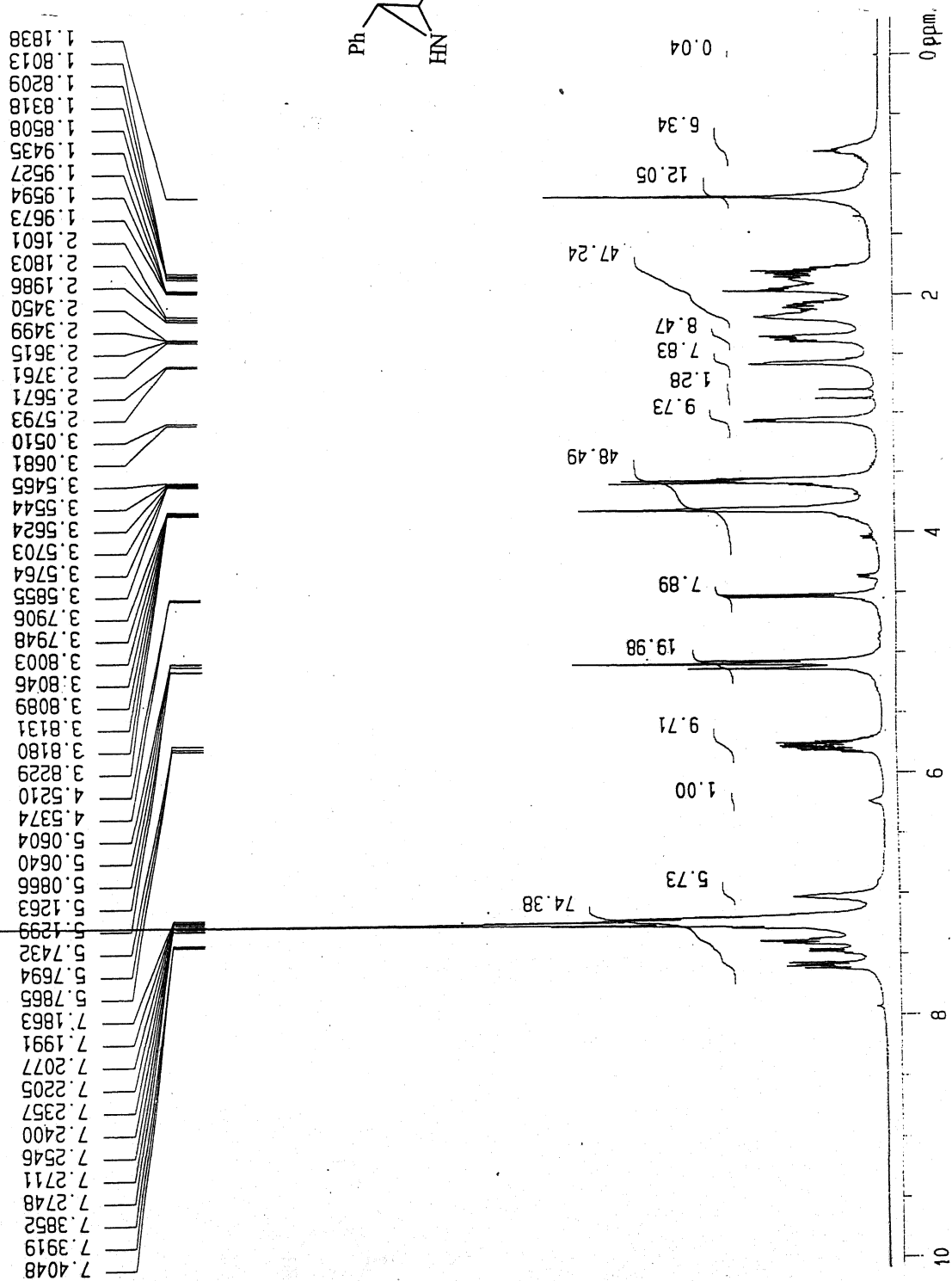
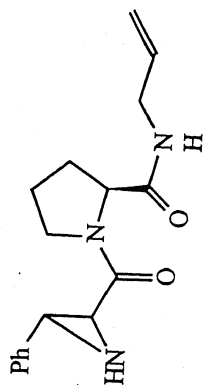
DMSO-d₆ addition
in steps of 0.05 mL
Sample Concentration
in CDCl₃ = 0.02 mM

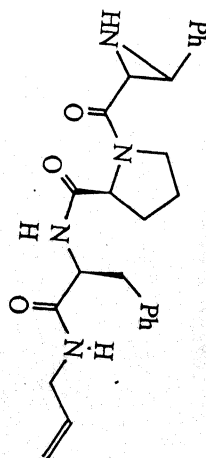
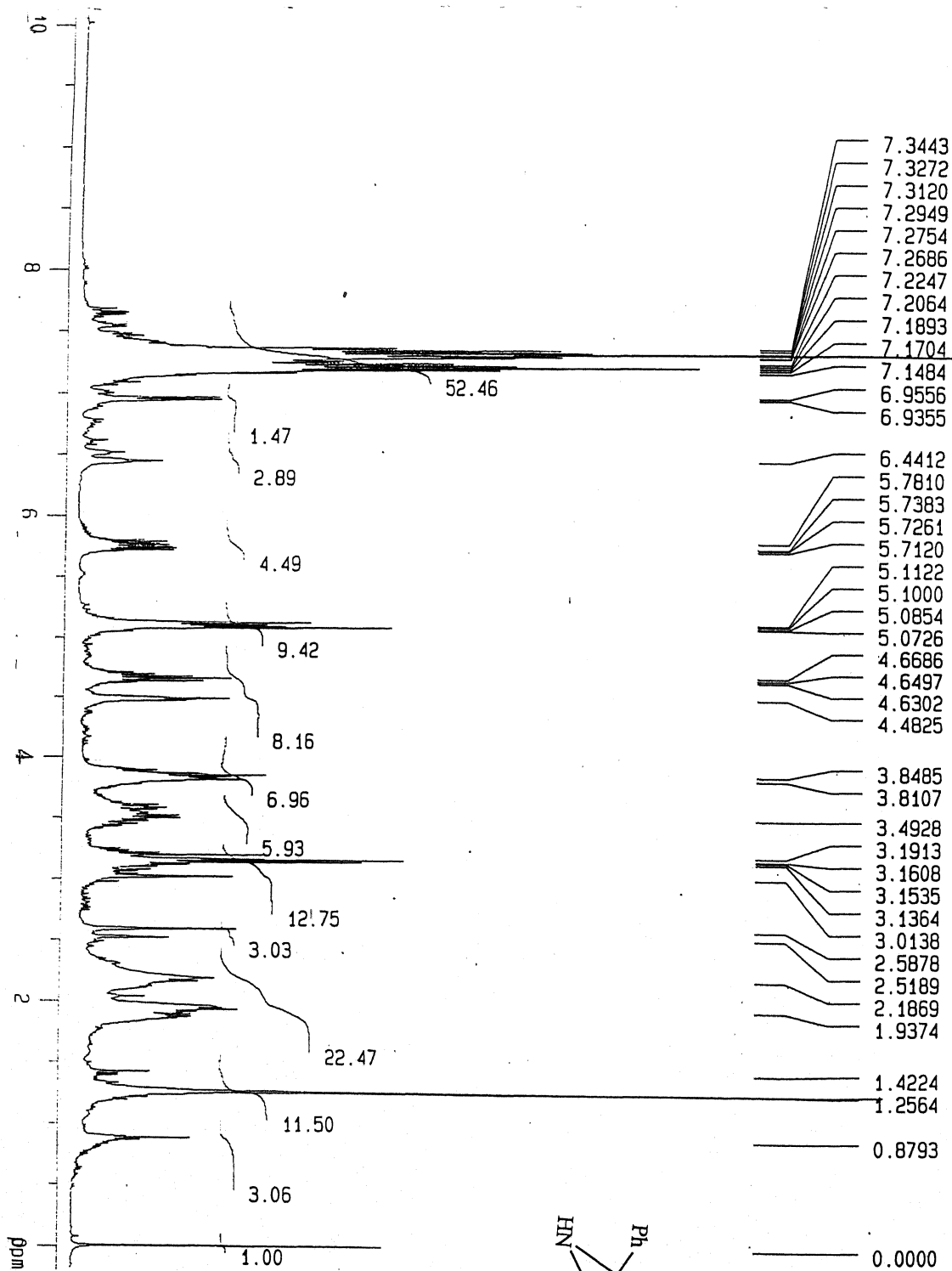


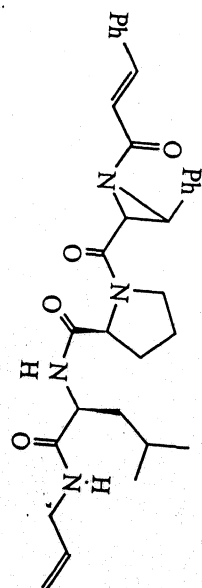
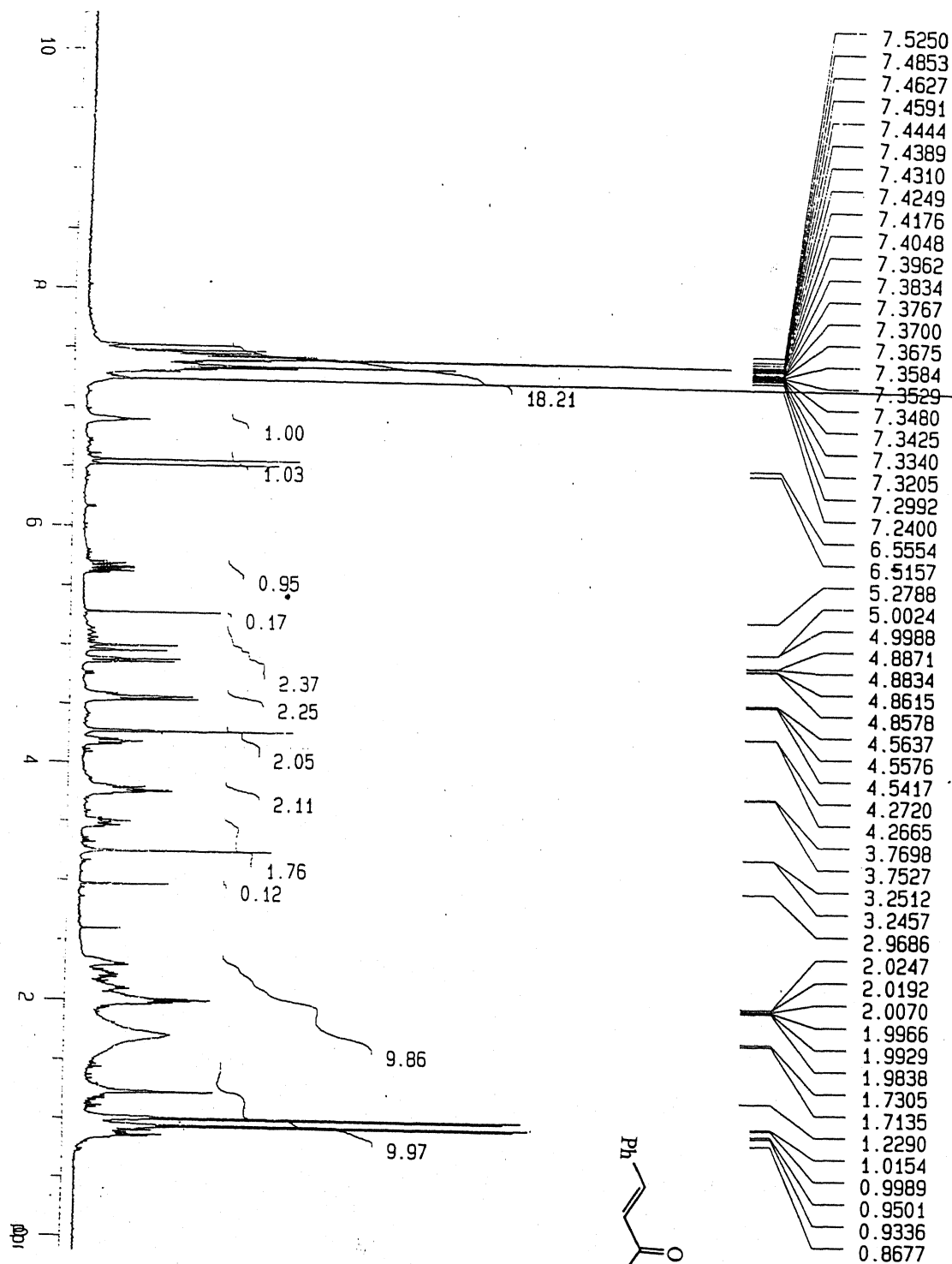


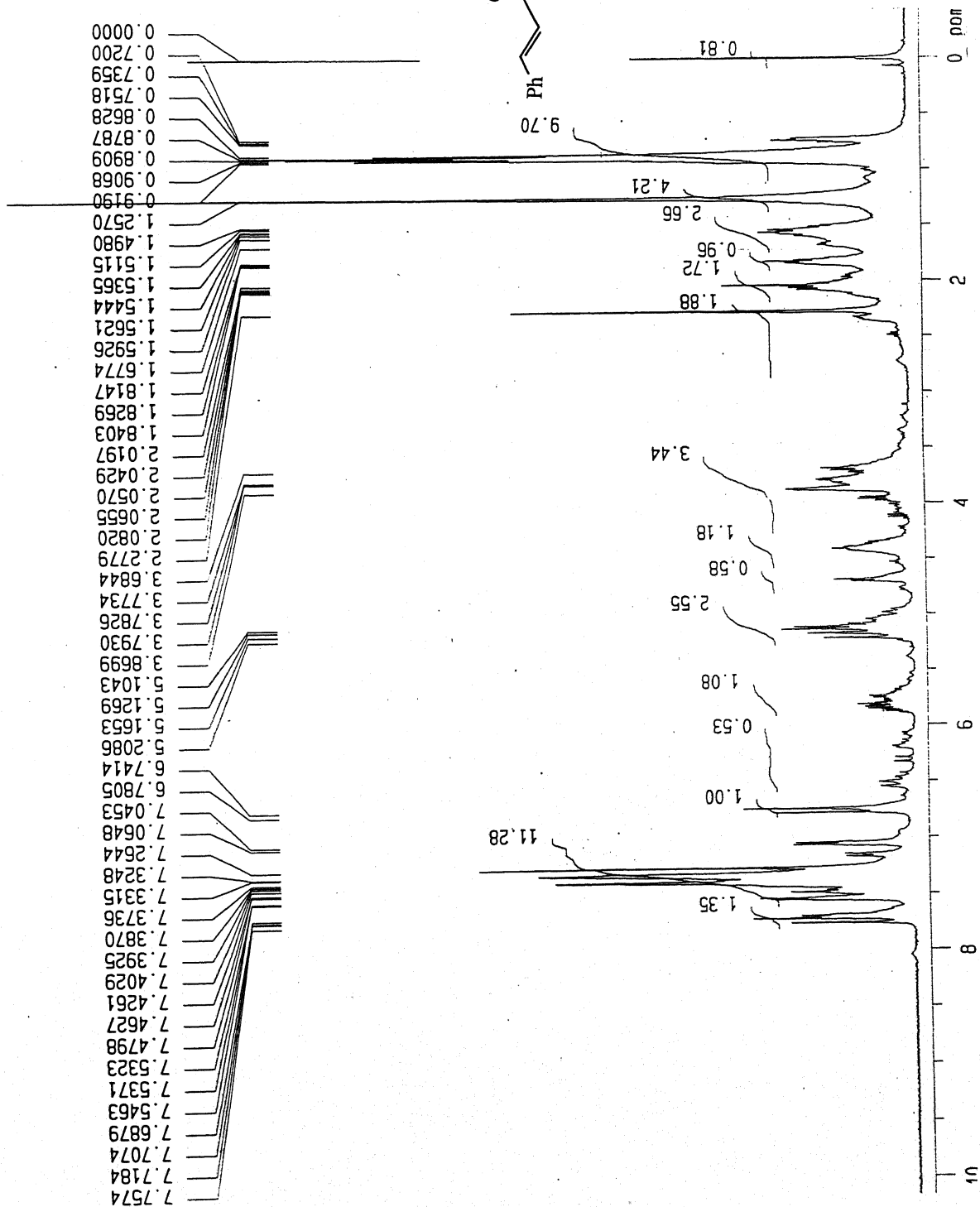
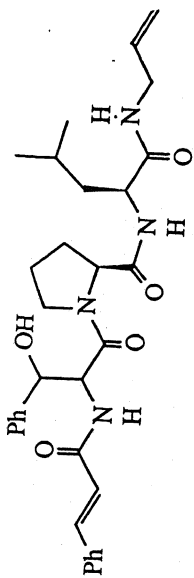
DMSO-d₆ addition
in steps of 0.05 mL
Sample Concentration
in CDCl₃ = 0.02 mM

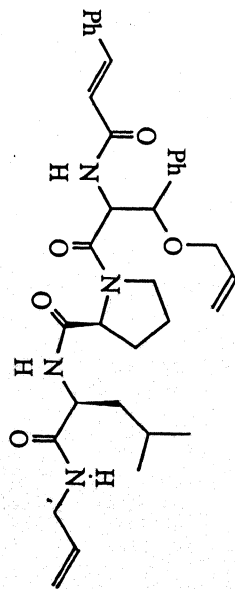
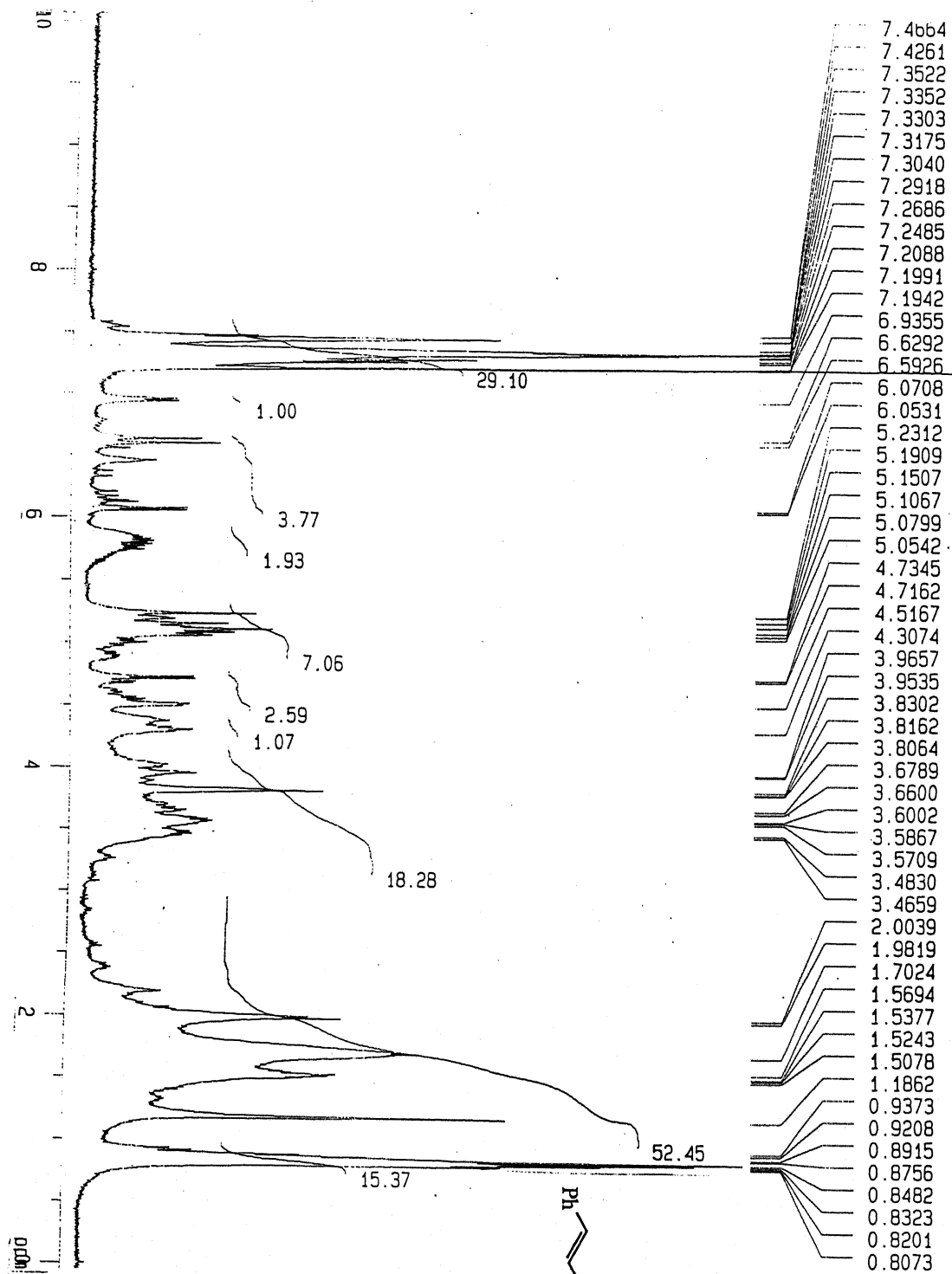


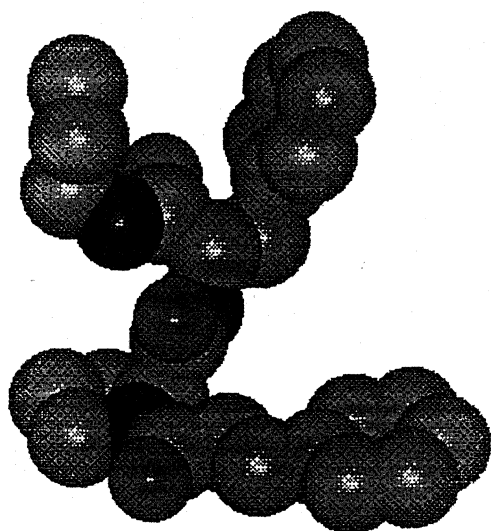




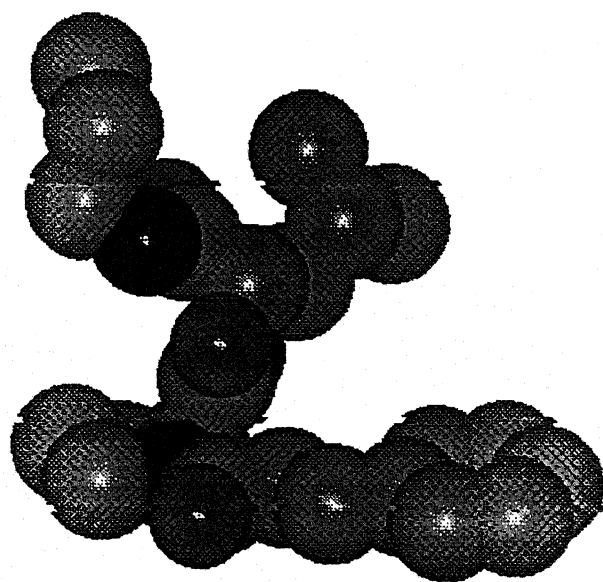




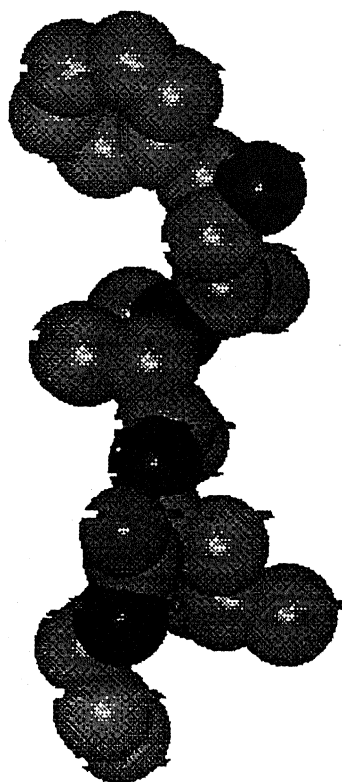




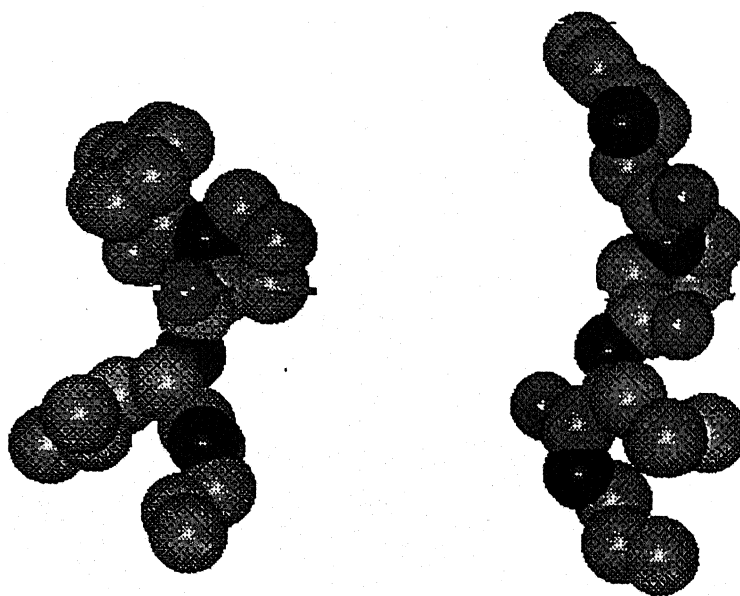
Allyl-N-cinnamoyl-Pro-Phe-amide



Allyl-N-cinnamoyl-Pro-Leu-amide



ϕ -Phe-Leu-Allylamide (ϕ = constrained conformational phenyl alanine)



Allyl-Phe-Pro-Leucine amide

Allyl- ϕ (Phe)-Pro-Leucine amide

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